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CONTENTS

PAGE

A Study of Forced Respiration: Experimental Production of Tetany. *Samuel B. Grant and Alfred Goldman* 209

A Study of the Carbohydrate Tolerance in Eck Fistula and Hypophysectomized Animals (Posterior Lobe Removal). Liver Function in the Metabolism of Sugars. *Conrad Jacobson* 233

The Gastric Response to Foods. XII. The Response of the Human Stomach to Pies, Cakes and Puddings. *Raymond J. Miller, Harry L. Fowler, Olaf Bergeim, Martin E. Rehfuss and Philip B. Hawk* 248

Segmental Activity in the Heart of the Limulus. *D. J. Edwards* 276

The Arterial Pressure Curve as Influenced by the Occlusion of Certain Vascular Areas and by Histamine. *D. J. Edwards* 284

The Artificial Production of Monsters Demonstrating Localized Defects as the Result of Injury from X-Rays. *W. M. Baldwin* 296

The Relation of the Epinephrin Output of the Adrenals to Changes in the Rate of the Denervated Heart. *G. N. Stewart and J. M. Rogoff* 304

The Effect of Acids, Alkalies and Salts on Catalase Production. *W. E. Burge* .. 364

On the Permeability of the Placenta for Adrenalin in the Pregnant Rabbit and Albino Rat. *Yoshitaka Shimidzu* 377

The Character of the Sympathetic Innervation to the Retractor Penis Muscle of the Dog. *Charles W. Edmunds* 395

Energy Expenditure in Household Tasks. *C. F. Langworthy and H. G. Barott* 400

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A STUDY OF FORCED RESPIRATION: EXPERIMENTAL PRODUCTION OF TETANY

SAMUEL B. GRANT AND ALFRED GOLDMAN

From the Physiological Department of Washington University

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INTRODUCTION

Tetany is a disease which has been subjected to a rather large amount of experimental study in recent years, the fact that its symptoms may be so readily produced by parathyroidectomy in animals, and the question of the relation of parathyroid tetany to idiopathic tetany in man, making it a particularly inviting field for experimental work. In the course of the work about to be described, it was found that all the essential symptoms of tetany could be produced in the human subject by forced respiration.

It was noted during the course of a series of experiments in which the subject was required to go through a period of increased respiration, that the urine collected at the end of this period was cloudy, and that the cloudiness was due to the fact that the urine was alkaline and contained precipitated phosphates. In explanation of these observations it was surmised that the overventilation, by washing out carbon dioxide of the blood, tended to make the blood more alkaline, and that in order to maintain a constant hydrogen ion concentration in the body, alkali was excreted by way of the kidneys. The present investigation was undertaken with the idea of determining this question.

METHODS

The experiments were carried out on human subjects according to the following plan. During the period of forced respiration the subject, with thoracic and abdominal pneumographs attached, was lying on his

back, and breathed as deeply as possible in time with a metronome at the rate of about 14 per minute. This was continued until symptoms of tetany developed, usually for from 15 to 60 minutes. The pneumographs recorded the depth of respiration on smoked paper, within sight of the subject, who was thus better able to maintain the deep breathing. In the later experiments, when the subjects had become accustomed to forcing respiration, the pneumographs were discarded. The metronome, however, was used throughout to prevent the rate of breathing from becoming involuntarily too slow.

The alveolar carbon dioxide tension was measured at the beginning and at the end of each experiment, the Plesch (1) bag method of collecting the air and the Marriott (2) colorimetric method of determining carbon dioxide tension being used. In some of the experiments the alveolar carbon dioxide tension was determined at intervals throughout the period of forced respiration, and afterwards until it had returned to normal.

The urine secreted during a measured period of time just before the experiment was collected, and also that for the period during which the deep breathing occurred, and in some cases at fixed intervals subsequently until the urine had returned to normal. Each specimen was analyzed as to hydrogen ion concentration, titratable acidity and ammonia content. The hydrogen ion concentration was determined by the method of Henderson and Palmer (3). The acidity was titrated with 0.1 N NaOH, using phenolphthalein as the indicator. Ammonia was determined by the Folin permutit (4) method.

Twenty cubic centimeters of blood were drawn with a syringe from the veins at the elbow just before and at the end of forced respiration, and the hydrogen ion concentration, carbon dioxide capacity and calcium content were determined. The hydrogen ion concentration was determined by the dialyzing and colorimetric method of Levy, Rowntree and Marriott (5). Samples of blood immediately upon being collected and before coming in contact with the air, were put into the collodion sacs and dialyzed. Specimens of oxalated whole blood were also used in several cases. The carbon dioxide capacity of the blood was determined by the method of Van Slyke (6), and calcium was determined by the Lyman (7) method, both whole blood and serum being used.

RESULTS

Tetany in forced respiration. Symptoms of tetany developed in each of twenty-four experiments, with the exception of the one control. The diagnosis was based on the presence of carpopedal spasm, Chvostek's sign, Trousseau's sign, Erb's sign of increased electrical irritability, and in one instance a tetanic convulsion.

Attention was drawn to this condition in the first experiment. The subject, S. G., breathed as deeply as possible for sixteen minutes, at the rate of 15 per minute. About ten minutes after the start his fingers began to tingle. At the end of fifteen minutes the muscles of his face felt stiff, and there was some difficulty in articulation. In the second experiment, A. G., subject, tingling in the fingers was noted. When at the end of forty minutes an attempt was made to determine the blood pressure, inflation of the arm band caused the hand immediately to go into a typical spasm of tetany. The fingers were flexed at the metacarpophalangeal joints; the second and third phalanges remained extended at the phalangeal joints; all the fingers, especially the thumb, were adducted and the hand was flexed at the wrist. The spasm could be voluntarily overcome by active motions, but as soon as these ceased the hand returned to the former position. Generally the spasm relaxed almost immediately after cessation of the forced breathing, but sometimes it persisted for as long as three minutes. In subsequent experiments the other signs and symptoms of tetany noted above were brought out.

Two subjects were used throughout the course of the experiments, and it was found that they differed somewhat in their reaction. Subject S. G. developed, after breathing deeply for about ten minutes, spasticity of the facial muscles. Chvostek's sign for tetany could then be easily elicited, i.e., tapping a branch of the facial nerve caused spasmodic contraction of the facial muscles. As the deep breathing continued this spasticity increased, until finally the muscles of the face, especially those about the mouth, contracted spontaneously, causing puckering of the lips and decided difficulty in articulation. In the meantime the hands had begun to tingle and feel numb, and finally, after the spasm of the face was well marked, they spontaneously went into the typical spasm described above. Chvostek's sign remained positive after forced respiration had ceased, and a slight reaction could be obtained at least twenty minutes after the end of the experiment. In subject A. G. the facial phenomenon was not nearly so marked, but the spasm of the hands was greater. He often noted a slight headache

soon after deep breathing started. In about fifteen minutes tingling of the hands began, and this gradually became more intense until the hands suddenly went into a very marked spasm. Trousseau's sign could usually be obtained several minutes before the onset of the spontaneous spasm. When the facial nerve was tapped only a slight twitching of the muscles occurred and this was usually not obtained until the carpal spasm was intense, and not always then. No spontaneous facial spasm occurred as in subject S. G. Spasm of the feet took place in two experiments in which A. G. was subject. The feet were extended, the first phalanges of the toes flexed and the second and third extended.

Tingling in the abdominal and thoracic muscles was noted by both subjects on several occasions, when the period of deep breathing was prolonged.

The electrical irritability of the muscles of the forearm, or Erb's sign, was tested in two experiments, and in both it was found that contraction occurred with a much smaller current at the end of deep respiration than before. The indifferent electrode was fastened to the upper part of the right arm, and the stimulating electrode was applied over the ulnar nerve just above the elbow, the same point of stimulation being used before and after forced breathing. The number of milliamperes of current necessary to cause contraction with the cathodal opening, cathodal closing, anodal opening and anodal closing stimuli before and at the end of deep respiration in experiment 18 were as follows:

Experiment 18

	C O C	C C C	A O C	A C C
Before deep respiration	5.5	2.5	2.5	2.0
	CC TETANY			
At end of deep respiration	1.25	0.5	1.25	1.25
In experiment 5 they were:				
Before deep respiration			3.5	1.8
At end of deep respiration			0.5	0.+

A complete tetanic convulsion occurred in experiment 18, in which A. G. was subject. No note of the exact rate of respiration was made but it was faster than usual. After breathing very deeply for thirty minutes he suddenly went into a complete tetanic convulsion. At the onset he involuntarily gave a loud, high-pitched scream, probably due

to contraction of the muscles of respiration and forcing of air out through contracted vocal cords. The entire body was rigid, all the muscles being contracted in tetanic spasm. The back was arched somewhat, and all extremities extended completely. Relaxation occurred within thirty seconds, and there was no further spasm. There were no ill effects following the convulsion.

The usual sequence of symptoms in the experiments is given in the following chart of a typical experiment, together with some of the experimental data.

Experiment 11. A. G., subject

TIME	
Before	Alveolar CO_2 tension, 40 mm.
	Urine pH = 4.9
	Blood pH = 7.4
	Plasma CO_2 capacity, 63.6 vol. per cent
0 minute	Starts deep breathing—12 per minute
10th minute	Slight headache
13th minute	Tingling in hands
16th minute	Trousseau's sign obtained. Slight Chvostek
17th minute	Both hands tingle markedly
20th minute	Spasm of both hands
21st minute	Alveolar CO_2 tension 20 mm.
22d minute	Blood collected
	pH=7.5
	Plasma bicarbonate 45.8 vol. per cent
23d minute	Spasm of feet. No facial spasm. Stops deep respiration
25th minute	Urine collected. pH=7.9

Changes in the blood, urine and alveolar air. Decided changes took place in the alveolar air, blood and urine as a result of the forced respiration. Alevolar carbon dioxide tension fell, the blood became slightly more alkaline, the urine became decidedly alkaline, the plasma bicarbonate was reduced, the ammonia excretion was diminished, and there was a slight increase in the calcium content of the blood.

Alveolar carbon dioxide. In fifteen experiments in which the alveolar carbon dioxide tension was measured it fell from an average of 42 mm. before to 21 mm. of mercury at the end of the period of deep breathing (see table 1). The fall was rapid at first and then became slower. With the termination of the period of forced breathing the tension rose at first rapidly and then more and more slowly, to almost reach normal in twenty minutes (see figs. 1, 2, 3, 4).

In experiment 17 the alveolar air was collected both by the Haldane (8) method, which gives alveolar air as determined by the tension of the gases in arterial blood, and the Plesch method, which gives alveolar air at the tension the gases are contained in venous blood. It was found that the difference in carbon dioxide tension as determined by these two methods was less at the termination of the period of exaggerated respiration than it had been at the beginning, as shown in table 2.

TABLE 1

EXPERIMENT	ALVEOLAR CO_2 TENSION		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	At end of deep respiration		
			minutes	
1	40	22	16	S. G.
2	35	18	50	A. G.
3	38	18	32	A. G.
4	43	22	15	S. G.
5	40	22	25	A. G.
6	40	25	25	S. G.
8	45	22	25	S. G.
9	41	24	26	A. G.
10	45	22	20	S. G.
11	40	20	23	A. G.
12	45	25	20	S. G.
13	37	20	28	A. G.
14	45	21	37	A. G.
15	45	20	23	S. G.
16	45	22	23	S. G.
Average.....	41.8	21	25.9	

TABLE 2

TIME AFTER START OF DEEP RESPIRATION	ALVEOLAR CO_2 TENSION (MM.Hg.)		DIFFERENCE IN TENSION
	Plesch	Haldane	
minutes	*mm.*	*mm.*	*mm.*
0	45	37	8
5.5	31	23	8
11.5	26	19	7
16.0	23	18	5
21.5	22	18	4

Hydrogen ion concentration of the blood. This was determined in five experiments, and the average fall was from pH 7.41 before to pH 7.57

at the end of deep breathing (see table 3). These determinations were made on fresh blood directly from the vein, before clotting occurred. In three experiments the hydrogen ion concentration of oxalated blood was determined. Loss of carbon dioxide to the air could not be so readily prevented when this was done, but the blood was placed in a narrow test tube from the syringe, corked, gently mixed with oxalate crystals, and then dialyzed at once in the deep narrow collodion sacs. The results were as in table 4. The hydrogen ion concentration of blood may be calculated from the carbon dioxide capacity of the plasma

TABLE 3

EXPERIMENT	pH OF BLOOD		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	At end of deep respiration		
			minutes	
9	7.40	7.60	26	A. G.
10	7.45	7.65	20	S. G.
11	7.40	7.50	23	A. G.
12	7.35	7.60	20	S. G.
13	7.45	7.50	28	A. G.
Average.....	7.41	7.57	23.5	

TABLE 4

EXPERIMENT	pH OF OXALATED BLOOD		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	At end of deep respiration		
			minutes	
11	7.45	7.65	23	A. G.
12	7.35	7.60	20	S. G.
13	7.35	7.50	28	A. G.

and the alveolar carbon dioxide tension, using Hasselbach's (9) formula. This was done in three experiments in which the required data had been obtained, and in each of these the blood was shown to have become more alkaline during deep respiration. In experiment 2 pH rose from 7.44 to 7.61, in experiment 12 from 7.34 to 7.48, and in experiment 13 from 7.40 to 7.55.

Plasma bicarbonate. The carbon dioxide combining power of the plasma was determined in four experiments. It fell from an average of 59.5 v.p.c. before to 44.9 v.p.c. at the end of deep respiration. The greatest fall was 17.8, the smallest 13.0 (see table 5).

TABLE 5

EXPERIMENT	CO_2 CAPACITY OF PLASMA		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before	At end of deep respiration		
	v.p.c.	*v.p.c.*	*minutes*	
11	63.6	45.8	22	A. G.
12	56.0	43.0	17	S. G.
13	52.9	38.7	27	A. G.
21	65.6	52.2	21	S. G.
Average.....	59.5	44.9	21.75	

Hydrogen ion concentration of urine. The urine specimen collected after forced respiration was alkaline to litmus in twelve of thirteen experiments. The determination of the hydrogen ion concentration showed, in the average of thirteen experiments, pH 5.2 in the specimens voided before the period of exaggerated respiration, and pH 7.4 in the specimens voided after this period (see table 6).

TABLE 6

EXPERIMENT	pH OF URINE		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	After deep respiration		
			minutes	
1	6.4	6.9	16	S. G.
2	4.7	7.1	50	A. G.
3	5.5	7.4	32	A. G.
4	4.8	7.3	15	S. G.
5	4.7	7.9	25	A. G.
6	5.0	7.1	25	S. G.
8	4.7	7.7	25	S. G.
9	5.5	7.2	26	A. G.
10	5.2	7.8	20	S. G.
11	4.9	7.9	23	A. G.
12	4.8	7.1	20	S. G.
13	5.8	7.7	28	A. G.
16	5.7	7.2	23	S. G
Average.....	5.2	7.4	25.2	

Titratable acidity of urine. In twelve experiments the acidity of the urine as titrated with 0.1 N sodium hydroxide, using phenolphthalein as the indicator, fell from an average of 7.54 cc. 0.1 N acid to 2.23 cc. per half-hour (see table 7).

TABLE 7

EXPERIMENT	TITRATED ACID		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Of before urine; cc. 0.1 N per ½ hour	Of after urine; cc. 0.1 N per ½ hour		
	cc. 0.1 N	*cc. 0.1 N*	*minutes*	
2	10.93	2.80	50	A. G.
3	8.70	1.75	32	A. G.
4	9.37	1.68	15	S. G.
5	10.54	2.36	25	A. G.
6	10.05	1.46	25	S. G.
8	4.59	0.75	25	S. G.
9	6.34	2.14	26	A. G.
10	3.53	1.24	20	S. G.
11	5.40	1.67	23	A. G.
12	5.80	2.07	20	S. G.
13	7.88	1.74	28	A. G.
16	9.72	3.05	23	S. G.
Average.....	7.54	2.23	26	

Ammonia excretion. The ammonia content of the urine was determined in twelve experiments. The average decrease was from 13.18 mgm. ammonia nitrogen per half-hour before, to 5.57 mgm. ammonia nitrogen after forced respiration (see table 8).

TABLE 8

EXPERIMENT	AMMONIA		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	In before urine; mgm. NH_3 nitrogen per half-hour	In after urine; mgm. NH_3 nitrogen per half-hour		
	mgm.	*mgm.*	*minutes*	
2	16.45	7.85	50	A. G.
3	13.40	4.74	32	A. G.
4	14.20	5.32	15	S. G.
5	16.50	5.85	25	A. G.
6	9.00	3.90	25	S. G.
8	11.74	4.28	25	S. G.
9	17.40	10.13	26	A. G.
10	8.00	3.93	20	S. G.
11	12.00	7.20	23	A. G.
12	10.20	4.50	20	S. G.
13	14.25	4.13	28	A. G.
16	15.00	5.00	23	S. G.
Average.....	13.18	5.57	26	

Calcium content of blood. Calcium was determined in whole blood in one experiment and in serum in three experiments, and in each case a small but definite increase in calcium content was found in the blood collected at the end of the period of deep respiration over that found in the blood before deep respiration. All determinations were run in duplicate, some in triplicate, and a blank was run in each case. The figures are given in table 9.

TABLE 9

EXPERIMENT	Ca CONTENT OF BLOOD		LENGTH OF PERIOD OF DEEP RESPIRATION	SUBJECT
	Before deep respiration	At end of deep respiration		
	mgm. per 100 cc.	*mgm. per 100 cc.*	*minutes*	
18 (Whole blood)	7.00	7.29	67	A. G.
19 (Serum)	11.81	13.19	51	S. G.
20 (Serum)	12.75	13.31	62	A. G.
21 (Serum)	12.84	13.44	21	S. G.

The blood taken from the veins at the end of deep respiration had a much brighter red color than normal venous blood.

Control experiments. It was necessary to show, *a*, that the intake of food was not influencing the results; and *b*, that the results could not be attributed to any factor other than the overventilation. In experiments 9, 10, 11 and 12 the subjects omitted breakfast on the morning of the experiment. There was no appreciable difference between the results in these experiments and any of the others except that the titratable acidity of the control urine specimens was less than in the other experiments.

In experiment 7 means were taken to prevent the subject from lowering the carbon dioxide content of the alveolar air. He breathed through a tube connected to a fifteen-liter bottle, while the observer at the same time breathed through a second tube connected to the bottom of the bottle. The resulting high carbon dioxide content of the air in the bottle prevented the subject from washing out the carbon dioxide from his blood, in spite of the forced respiration. Under these conditions the alveolar carbon dioxide tension was slightly higher at the end of the experiment than it had been before. The acidity and ammonia content of the urine increased slightly, rather than decreasing as in the other experiments, and the hydrogen ion concentration of the urine remained about the same. The figures are given in table 10.

No symptoms of tetany occurred in this experiment. The subject became very slightly cyanosed, and after the experiment was dyspnoeic for a few minutes. Breakfast was omitted on the morning of the experiment.

TABLE 10

A. G. subject. Deep breathing 17 minutes

URINE pH		URINE ACID (CC. 0.1 N)		NH_3 NITROGEN (MGM.)		ALVEOLAR CO_2	
Before	After	Before	After	Before	After	Before	After
4.6	4.8	7.79	8.27	11.2	13.3	38	40

Four of the experiments may now be taken up in detail in order to illustrate more concisely the exact method of procedure and the uniformity of the results.

Experiment 12. S. G., subject

TIME	
	Alveolar CO_2 tension = 45 mm.
	Urine pH = 4.8
0 minutes	Blood pH = 7.35
	Plasma CO_2 capacity = 56 v.p.c.
10 minutes	Starts forced respiration
20 minutes	Chvostek's sign obtained. Alveolar CO_2, 28 mm. Tingling in fingers
23 minutes	Spasm of facial muscles—Chvostek's sign marked. Trousseau's sign positive
27 minutes	Blood collected pH = 7.60 Plasma CO_2 capacity = 43 v.p.c.
30 minutes	Alveolar CO_2 tension = 25 mm. Stops forced respiration
40 minutes	Urine collected pH = 7.1
45 minutes	Alveolar CO_2 tension = 33 mm.
55 minutes	Alveolar CO_2 tension = 37 mm.
1 hour 10 minutes	Urine collected pH = 5.0
1 hour 15 minutes	Alveolar CO_2 tension = 39 mm.
2 hours 10 minutes	Urine collected pH = 5.0
2 hours 20 minutes	Alveolar CO_2 tension = 40 mm.

The graph of this experiment (fig. 1) shows the synchronous changes in alveolar air, blood and urine. Time in minutes is plotted on the horizontal axis, and alveolar carbon dioxide tension in millimeters of mercury, plasma carbon dioxide capacity in volume per cent, hydrogen

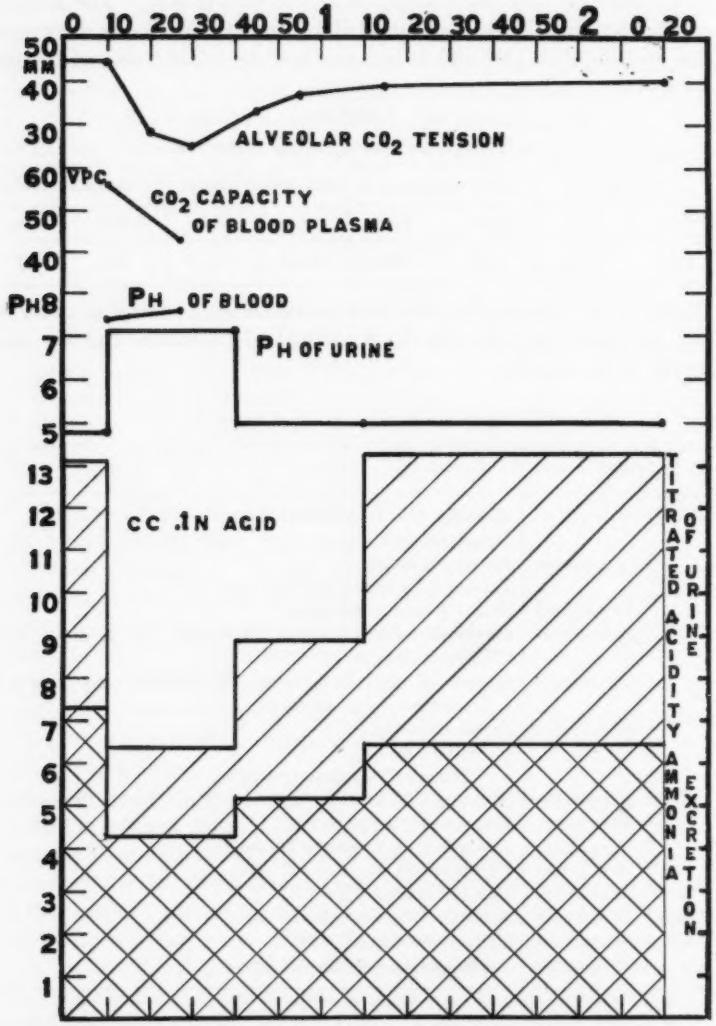

Fig. 1. Experiment 12. Forced respiration begins at 10 minutes and ends at 30 minutes.

ion concentration of blood and urine in terms of pH, and ammonia excretion and titrated acidity of urine per half-hour in cc. .1N solutions on the perpendicular axis. Deep breathing starts at 10 and ends at 30 minutes. As the alveolar CO_2 tension falls, the CO_2 capacity of the plasma falls, hydrogen ion concentration of blood and urine fall, excretion of ammonia decreases, and titrated acidity of urine falls. After deep breathing ceases, alveolar CO_2 tension rises, and corresponding changes occur in the urine.

Experiment 13. A. G., subject

TIME	
0 minute	Alevolar CO_2 tension = 37 mm.
	Urine pH = 5.8
	Blood pH = 7.45
	Plasma CO_2 capacity = 52.9 v.p.c.
10 minutes	Starts forced respiration
25 minutes	Slight headache
30 minutes	Alveolar CO_2 tension = 23 mm.
31 minutes	Tingling in right foot. No Chvostek
32 minutes	Hands tingle
33 minutes	Tingling increased
34 minutes	Trousseau's sign markedly positive
35 minutes	Both hands in spontaneous spasm
36 minutes	Slight spasm of feet
37 minutes	Blood drawn
	pH = 7.50
	Plasma CO_2 capacity = 38.7 v.p.c.
	Spasms of hand more intense
38 minutes	Stops forced respiration
40 minutes	Alveolar CO_2 tension = 20 mm.
42 minutes	Urine collected pH = 7.7
	Temporary dizziness and trembling of hands on standing up after forced respiration
45 minutes	Alveolar CO_2 tension = 28 mm.
50 minutes	Alveolar CO_2 tension = 32 mm.
1 hour	Alveolar CO_2 tension = 37 mm.
1 hour 23 minutes	Urine collected pH = 6.9
1 hour 50 minutes	Urine collected pH = 5.0

In the graph of this experiment (fig. 2) the same changes are shown as in figure 1, with the exception that the titrated acidity of the urine is not charted. Deep breathing starts at 10 and ends at 38 minutes. The third specimen of urine after forced breathing is more acid than the control urine specimen.

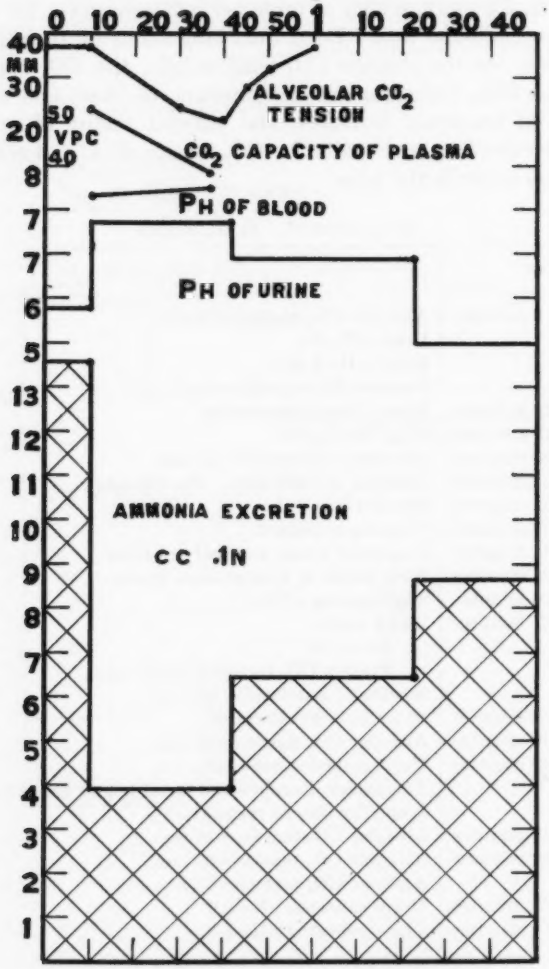

Fig. 2. Experiment 13. Forced respiration begins at 10 minutes and ends at 38 minutes.

Experiment 16. S. G., subject

TIME	
30 minutes	Urine collected pH=5.0
1 hour	Urine collected pH=5.7
1 hour 5 minutes	Alveolar CO_2 tension=45 mm.
1 hour 7 minutes	Starts forced respiration
1 hour 12 minutes	Alveolar CO_2 tension=31 mm.
1 hour 18 minutes	Alveolar CO_2 tension=26 mm.
1 hour 22 minutes	Tingling in hands
1 hour 23 minutes	Marked tingling in hands. Alveolar CO_2 tension=23 mm.
1 hour 26 minutes	Carpal spasm
1 hour 28 minutes	Alveolar CO_2 tension=22 mm.
1 hour 30 minutes	Stops forced respiration
	Urine collected pH=7.2
2 hours	Urine collected pH=6.8
2 hours 45 minutes	Urine collected pH=5.8
3 hours 45 minutes	Urine collected pH=4.8
4 hours 15 minutes	Urine collected pH=5.5

Experiment 4. S. G., subject

TIME	
20 minutes	Alveolar CO_2=43 mm.
25 minutes	Urine collected pH=4.8
30 minutes	Starts forced respiraton
38 minutes	Tingling and numbness in hands
40 minutes	Headache
41 minutes	Rigidity of facial muscles. Chvostek markedly positive, right and left
42 minutes	Carpal spasm both hands
44 minutes	Abdominal and thoracic muscles begin to tingle. No spasticity felt by observer
45 minutes	Stops forced respiration
	Aveolar CO_2 tension=22 mm.
55 minutes	Urine collected pH=7.3
	Chvostek weakly positive
	Alveolar CO_2 tension=32 mm.
1 hour 7 minutes	Chvostek negative
1 hour 15 minutes	Alveolar CO_2 tension=40 mm.
1 hour 25 minutes	Urine collected pH=4.8
1 hour 35 minutes	Alveolar CO_2 tension=43 mm.
2 hour 25 minutes	Urine collected pH=4.7
3 hour 25 minutes	Urine collected pH=5.2

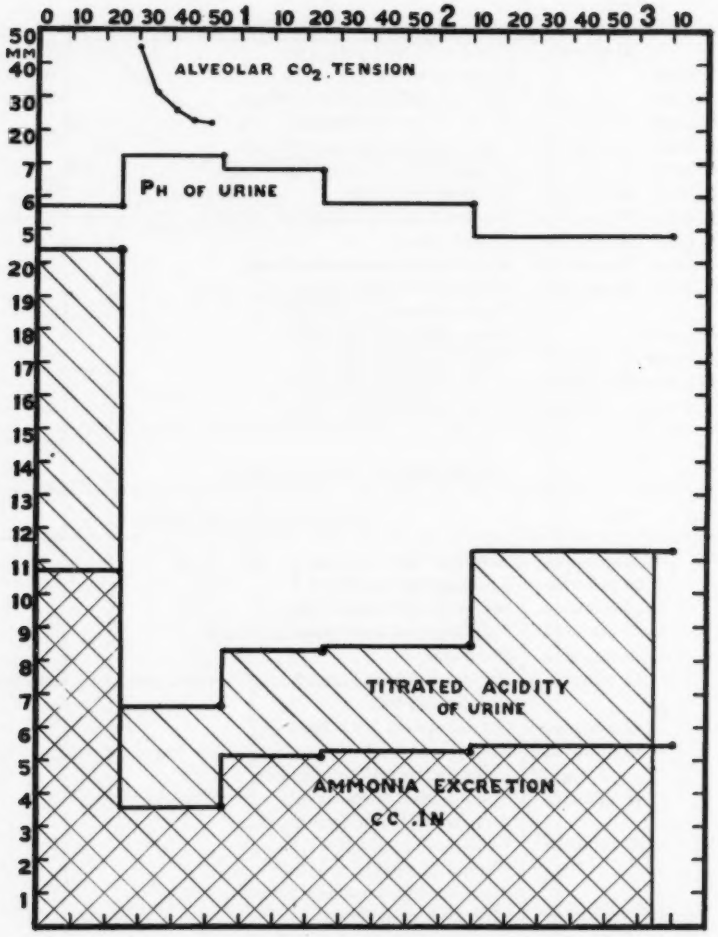
0 10 20 30 40 50 1 10 20 30 40 50 2 10 20 30 40 50 3 10
MM
ALVEOLAR CO_2 TENSION
PH OF URINE
TITRATED ACIDITY OF URINE
AMMONIA EXCRETION CC. .IN

Fig. 3. Experiment 16. Forced respiration begins at 30 minutes and ends at 53 minutes.

Figure 3 is a graph of experiment 16. Deep breathing starts at 30 minutes and ends at 53 minutes. The fall in alveolar CO_2 tension is very marked at first and then gradually becomes smaller as the low

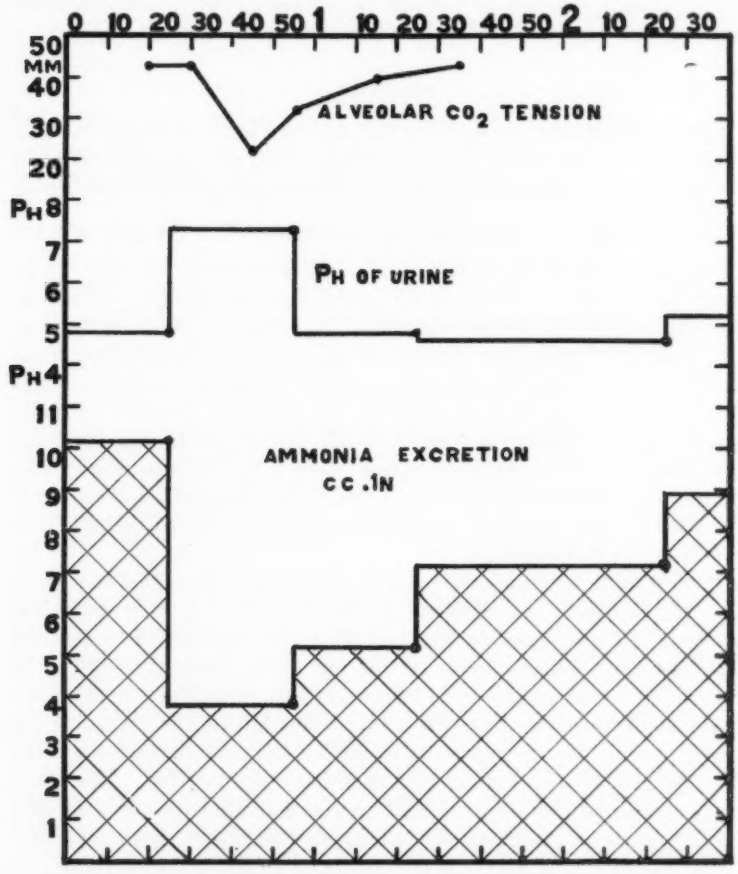

Fig. 4. Experiment 4. Forced respiration begins at 30 minutes and ends at 45 minutes.

level is reached. The urine pH reaches its control level 1 hour and 15 minutes after the forced respiration, while the specimen collected 2 hours 15 minutes after is more acid than the control one. The other changes are similar to those shown in figures 1 and 2.

The changes in experiment 16 (fig. 4) are similar to those described in the previous ones. The deep breathing begins at 30 and ends at 45 minutes. The return of the alveolar CO_2 tension to the control level is shown, 60 minutes elapsing before this occurs. The ammonia does not reach the control level in 2 hours and 30 minutes.

DISCUSSION

Forced respiration, by washing out carbon dioxide from the alveoli, reduces the carbon dioxide content of the blood and so tends to make the blood more alkaline than normal. This reduction of carbon dioxide in the blood disturbs the $\frac{H_2CO_3}{NaH\ CO_3}$ ratio upon which the hydrogen ion concentration depends, and on the reëstablishment of this ratio, $NaH\ CO_3$ passes out of the blood plasma into the tissues, and a considerable portion is excreted by way of the kidneys. Apparently, CO_2 may be washed out of the blood more rapidly by overventilation, than $NaH\ CO_3$ is decreased by excretion or other means, for on this assumption alone is it possible to account for the increased alkalinity actually found in blood during deep respiration. The diminution in ammonia formation is a further evidence of the effort of the body to compensate for the increased alkalinity of the blood. The lowered urine acidity is due partly to the increased alkali excretion, but probably largely to a retention of acid radicals.

In view of these considerations it seems logical to conclude that there is a true condition of "alkalosis" in the body during overventilation; the body fluids are actually more alkaline than normal. It is realized that the term "alkalosis" is a poor one, but we are using it strictly in the sense of an actual change in hydrogen ion concentration. In this condition of alkalosis we have an actual reduction of the "alkaline reserve," due to the elimination of $NaHCO_3$ from the body fluids in an effort to maintain a constant hydrogen ion concentration. The coexistence of a more alkaline blood and a reduced alkaline reserve is another example of the inadequateness of the terms "alkalosis" and "acidosis."

The question now arises, is it possible to establish an etiological relationship between this condition of alkalosis and the tetany which develops during forced respiration? This may be discussed in connection with the views as to the cause of tetany that are held at the present time, the two principal ones of which are, first, that it is due to a calcium deficiency in the blood; and second, that it is due to a disturbance of the acid-base equilibrium in the body.

The literature contains some references to this problem. Thus, Wilson, Stearns and Thurlow (10) and Wilson, Stearns and Janney (11) have found that in parathyroidectomized dogs, just before an attack of tetany, a period of "alkalosis" develops, which is neutralized by the acid substances that are produced during tetany. They base their conclusion that an "alkalosis" obtains on the study of the dissociation constant of the oxyhemoglobin, the CO_2 content of alveolar air, the hydrogen ion concentration of blood, the ammonia content of urine and the total acidity and hydrogen ion concentration of urine. Injections of acid and also of calcium salts relieve the tetany. They state that possibly part of the beneficial action of the calcium salts may be due to a relative increase in acid radicals caused by their administration. This might be brought about by the formation of $Ca_3(PO_4)_2$ from the carbonates, thus liberating HCl.

McCann (12) finds after parathyroidectomy (and following operations on the stomach which exclude the acid secreted from the duodenum) that there is a marked increase in the CO_2 combining power of the plasma, coincident with the development of tetany and concludes that tetany is a condition of alkalosis in which a disproportion between the rates of secretion of acids and alkalies by the gastro-intestinal tract may be a factor. He points out that clinically gastric tetany is most apt to occur in those cases in which there is some pyloric obstruction, so that the acid gastric secretion does not pass into the duodenum to excite the alkaline intestinal secretion, and that this might be expected to leave the blood with an excess of basic substances. MacCallum (13) and his coworkers in a paper just published state that when the pylorus of a dog is occluded, and only distilled water is introduced into the duodenum, the gastric mucosa continues to secrete hydrochloric acid. At the same time the alkali reserve increases and tetany-like symptoms appear. This can be prevented by the introduction into the duodenum of sodium chloride or the symptoms may be made to disappear by the intravenous injection of sodium chloride. Injection of hydrochloric acid is not so successful. Calcium determinations by a dialyzing method showed no change in the calcium content of plasma. The authors think that possibly the disturbed equilibrium of acids and bases in itself is the cause of the symptoms.

Cases of tetany following therapeutic administration of sodium bicarbonate have been reported. Howland and Marriott (14) cite three cases in children, all of which were associated with a marked diminution of the calcium content of the serum. Harrop (15) reports a case in an

adult who developed tetany after the intravenous injection of sixty grams of sodium bicarbonate. There was no associated diminution in calcium.

Binger (16) has shown that when sodium phosphate in sufficient quantity and at the right reaction is injected into dogs intravenously, symptoms of tetany result, associated with a marked decrease in the calcium content of the serum. But if the solution injected has a hydrogen ion concentration greater than 10^{-6}, no tetany occurs even though there is a diminution in calcium.

Ammonia poisoning produces symptoms strikingly similar to those of parathyroid tetany. Marfori (17) found after intravenous injections of the carbonate, lactate and tartrate of ammonia in dogs, tremors, muscle twitchings, even tetany and opisthotonus, and irregular respiration, vomiting, etc. MacCallum and Voegtlin (18) found increased ammonia content of the blood in parathyroid tetany as did also Carlson and Jacobson (19) in their earlier work. Later, however, using improved methods, Carlson and Jacobson could find no change in ammonia content of blood in parathyroidectomized dogs.

The fact that increased respiration is associated with changes in the reaction of the urine had been previously noted by Hasselbach (20). He found that when the alveolar CO_2 tension was raised a few millimeters by the influence of morphine on the respiratory center, the urine became very slightly more acid, and that when the alveolar CO_2 tension was decreased by stimulation of respiration by light baths, the urine became barely perceptibly more alkaline.

After the present work had been started, Leathes (21) published a paper in which it was stated that increasing the respiration voluntarily for from one-half to one hour increased the alkalinity per cent of the urine from about 35 per cent to about 90 per cent. There was no mention made of symptoms of tetany or other changes during deep respiration.

Yandell Henderson and Haggard (22) found a great reduction of CO_2 capacity of blood plasma of dogs in ether hyperpnoea and in excessive pulmonary ventilation by artificial respiration, together with a concomitant fall in blood pressure. They state that when the CO_2 capacity of plasma falls below 33 to 36 v.p.c., the process becomes irreversible, and the animal dies in shock. The lowest point to which CO_2 capacity of plasma fell in our work, in those experiments in which it was determined, was 43 v.p.c. Symptoms of tetany are not mentioned in their paper.

Stein (23) in discussing tetany as a sequel of gynecological operations cites fifteen cases in which it occurred. In four of these the symptoms of tetany occurred during the operation, and disappeared quickly without recurrence later. In three cases the tetany occurred during light ether anesthesia, one of these as narcosis was being induced, while the patient was still conscious. In the fourth case tetany occurred after the removal of eight liters of fluid from the peritoneal cavity by paracentesis, and lasted for fifteen minutes. It seems not unlikely that tetany in these instances may have been due to overventilation.

McClendon (24) has studied the effect of hydrogen and hydroxyl ions on the pulsation rate of the jelly fish and the heart of the conch, and finds that the activity may be inhibited by increasing H-ions in the surrounding fluid or perfusing liquid, as the case may be, and augmented by increasing the OH-ions. Biedermann (25) found that the heightened excitability of voluntary muscle produced by NaCl solution is greatly increased by the addition of Na_2CO_3, the muscle then contracting rhythmically and exhibiting a striking increase of response to artificial stimuli. Strong solutions of $NaSO_4$ and dilute NaOH act in a similar manner but to a less degree. He seems to attribute this to a specific effect of the sodium ion, but it may be that part of the action is due to the alkalinity of the solutions in the case of Na_2CO_3 and NaOH.

Recently a number of investigators have come to regard tetany as being due to a decrease in the calcium content of the blood. Thus MacCallum and Voegtlin (18) and MacCallum and Vogel (26) have shown that in the tetany of parathyroidectomized dogs there is a marked diminution of blood calcium. Howland and Marriott (27) have found that in infantile tetany the calcium content of the serum is greatly reduced, the average being about 5.6 mgm. per 100 cc. compared to 10 or 11 mgm. in the normal child. The administration of calcium promptly relieved the symptoms of tetany. On the other hand we have found in tetany of forced respiration no diminution of calcium content of the blood or serum. There is, as a matter of fact, a slight increase in calcium in every instance in blood specimens taken at the onset of tetany over those obtained under normal conditions of respiration. This may not necessarily mean that calcium plays no definite part in the production of the tetany of increased respiration. Thus Barille (Wells, 28) states that a decrease of CO_2 in the blood results in precipitation of calcium. And Marriott (29) has shown that when artificial blood is brought into equilibrium with CO_2 at 30 mm. Hg. tension, calcium is precipitated in a perceptible cloud. The possibility must

therefore be considered that when the CO_2 of blood is reduced by overventilation a portion of the calcium is "precipitated" or in some way rendered inactive, though still present in the circulating blood. On the basis of this supposition the increase of calcium which we have found might be interpreted as being due to a compensatory mechanism on the part of the body to make up for a loss of active calcium. The calcium in our subjects was not precipitated in the blood, however, for centrifuging the serum specimens did not affect the analysis. It is conceivable, however, that the calcium is "precipitated" as very minute particles which are held in solution by the colloids of the serum, in which case centrifuging might have no effect on the calcium present. A further attempt to determine whether calcium plays a part in the production of tetany of overventilation will be made in a future investigation.

It may not be inappropriate to refer in this connection to the hypothesis advanced by Stoeltzner (30) attributing tetany to an accumulation of calcium in the blood. He found support for his views in some observations on the electrical reactions of peripheral nerves in infants after the administration of calcium. But these observations of Stoeltzner's have failed of confirmation.

Tetany occurs under so many circumstances that it probably cannot be attributed to a single etiological factor. It is more likely that it must be regarded as a symptom complex, and possibly any condition that heightens the irritability of peripheral nerves may cause it. Observation seems to indicate that alkalosis may be such a cause, and it probably is the underlying factor in the tetany that develops as a result of forced respiration.

SUMMARY

1. Forced respiration causes symptoms of tetany to occur in the human subject; these include carpopedal spasm, Chvostek's sign, Trousseau's sign, Erb's sign, and in one instance a tetanic convulsion.

2. As a result of the fall of alveolar CO_2 tension produced by overventilation, there is a reduction in the hydrogen ion concentration of the blood, a reduction of the CO_2 capacity of plasma, a change in the reaction of the urine to the alkaline side, a decreased excretion of ammonia, and a slight increase in the calcium content of the serum.

3. The underlying factor in the tetany of forced respiration is the alkalosis.

We wish to express our deep appreciation of the invaluable help and interest in this work given us by Dr. Joseph Erlanger.

We are also indebted to Dr. P. A. Shaffer and Dr. W. McK. Marriott for their kindly interest throughout the work.

Note. After the completion of this paper, several important references bearing upon our results were found in a journal which had been temporarily lost from the library. H. M. Vernon (31) in some observations on the production of prolonged apnoea in man says, "I found that after about 6 minutes of forced breathing the muscles of my hands passed into a condition of tonic rigidity, and for the first one and one-half minutes of the subsequent apnoea they were completely paralyzed. My other muscles were apparently unaffected." In no case in our experiments were the hands paralyzed, for we have always been able to voluntarily overcome the spasm, and this is also true of the spasm of idiopathic tetany.

Yandell Henderson (32) had a large number of men undergo voluntary hyperpnoea for short periods of time (usually 45 to 90 seconds) and makes the following statement. "Several of the subjects after vigorous hyperpnoea for 1 or 2 minutes, experienced in varying degrees the phenomenon mentioned by Vernon. . . . Even more common in our experience is a prickling sensation in the legs and arms, or in some cases in the entire body and face, somewhat similar to a hand or foot 'asleep.'" In one case, after vigorous hyperpnoea for two minutes a "shivering fit" came on, similar to that seen in a chill, and involving apparently all the muscles.

Hill and Flack (33), in determining the effect of oxygen inhalation on muscular work, had men undergo voluntary hyperpnoea for short periods. They observed feelings of numbness in the limbs, with a spastic state of the hands. One subject felt "twitching of the facial muscles." In another the "mouth was felt pursed up in the form of an O, so that speaking became difficult," and the muscles surrounding the eyes seemed contracted, so that the eyes were difficult to open fully.

Apparently none of these investigators realized that the phenomena observed in forced breathing are identical with those seen in tetany, and no effort was made to determine the factors upon which they depend.

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A STUDY OF THE CARBOHYDRATE TOLERANCE IN ECK FISTULA AND HYPOPHYSECTOMIZED ANIMALS (POSTERIOR LOBE REMOVAL)

Liver Function in the Metabolism of Sugars

CONRAD JACOBSON

From the Peter Bent Brigham Hospital, Boston, Mass., and the Hunterian Laboratory of Surgical Research, Johns Hopkins Medical School, Baltimore, Md.

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In the course of some studies in the Hunterian Laboratory on the sugar tolerance of animals deprived of the hypophyseal gland (1) it was shown that there was a temporary lowering of the assimilation limit for saccharose (the sugar which was primarily used for reasons then given), often with spontaneous mellituria—usually dextro-rotatory, occasionally laevo-rotatory—followed by a subsequent rise to above normal. It was observed at the same time that this artificially raised tolerance as well as the normal tolerance of animals (cat, dog and rabbit) could be lowered occasionally to the point of spontaneous glycosuria by the administration, intravenously or by mouth, of hypophyseal posterior lobe extracts. It was assumed and has been since proven that the administration of the extract had a glycogenolytic effect and was accompanied by a hyperglycemia. In the further investigation of this subject, some efforts were made to determine the source of this glycogen. This was first attempted by staining methods—Best's Carmine of the liver—before and after discharge of the glycogen either by Bernard's Piqûre, by stimulation of the hypophysis or by giving hypophyseal or adrenal extracts. Though a lessening in the glycogen content of the cells could be demonstrated, the cells were never found entirely free from glycogen and the method had to be abandoned. Marked fatty changes in the liver had also been noted in some of the hypophysectomized animals and the suggestion arose that these changes might produce modifications in the storage capacity of the liver for glycogen and for carbohydrate metabolism. It was therefore decided to use an Eck fistula animal and to note what effect the shunting of the

liver from the portal circulation would have upon the sugar tolerance both before and after hypophysectomy.

The Eck fistula operation was first successfully performed on animals by von Eck in 1877 (2) and repeated with equal success by Stolnikow in 1882 (3). It consisted essentially of making an anastomosis of the portal vein to the vena cava with subsequent ligation and more recently, double ligation and transection of the portal vein near the liver hilus. In such a condition the blood supply of the liver is maintained by the intact hepatic artery, but the blood coming from spleen, pancreas, stomach and intestines, carrying with it the products of gastro-intestinal and splenic activity, is carried into the vena cava instead of to the liver and thus directly into the venous circulation. The purpose of the original operation was a clinical one, that of furnishing drainage for the abdominal fluid, ascites, incident to a cirrhosis of the liver, and neither of these two original investigators did any reliable work on changes in the metabolism. The operation has never proven successful when applied to human subjects both because of the operative difficulties and of the danger of subsequent toxic effects. It has, however, on repeated occasions been of extreme value in physiological investigations regarding carbohydrate metabolism and liver function.

In 1893 Hahn, Massen, Nencki and Pawlow (4), working with Eck fistula dogs, showed that such animals fed on a meat diet developed marked toxic effects, usually ending in the death of the animal. There was noted a definite decrease in the urinary output of uric acid and ammonia and a large increase in carbamate. On injecting the latter substance into normal and Eck fistula animals, they were able to produce similar toxic effects and concluded that accumulation of carbamate was the causal factor of the toxic symptoms. Rothberger and Winterberg in 1895 (5) verified the work of Pawlow *et al*, but could not satisfy themselves that carbamate was the toxic agent, concluding indefinitely that the toxic effect was caused by some substance which was removed by the liver under normal conditions but which, under the altered conditions of Eck fistula, accumulated in the blood. Nencki, Pawlow and Zaleski in 1896 (6) from experimental evidence concluded that ammonia was the cause of the toxic symptoms and not the carbamate. However in 1897 Nencki and Pawlow (7), by further experimentation with Eck fistula animals in which large portions of the liver were either removed or thrown out of function by ligation of the hepatic artery, concluded that the extreme toxic symptoms were far out of proportion to the increase of ammonia and could not consider the latter

as the main causal factor. Popelski in 1898 (8) showed that the Eck fistula dog had a low carbohydrate tolerance and that an alimentary glycosuria resulted when such an animal was fed large quantities of glucose. Sachs in 1898 (9), working with frogs whose livers had been extirpated, showed that they had a lower tolerance for laevulose than normal intact control animals, a finding which could not be confirmed with dextrose, galactose or arabinose. In 1901 Strauss (10), working with Sachs's findings as a rationale, introduced the laevulose test for hepatic insufficiency. In 1902 Schultz and Miller (11) had the opportunity of closely observing a clinical case of portal embolus at the liver hilus and were unable to find any definite untoward symptoms except that of ascites, reporting no pathological findings in urine. In 1907 Filippi (12) showed that there was a lowered tolerance for carbohydrates in dogs subsequent to Eck fistula operation. He showed that such animals possess an increased muscle glycogen content characteristic of over-nutrition and a low liver glycogen content characteristic of inanition. He inferred that the muscles could form glycogen independently and that the liver was neither specific for nor indispensable for carbohydrate metabolism. Hawk in 1908 (13), from feeding experiments, showed that quite extreme toxic effects were produced by feeding meat extracts (especially Liebig's extracts of meat) in conjunction with the meat diet. He was, however, unable to produce any such toxic symptoms by the introduction of carbamate nor was he able to produce glycosuria on carbohydrate feeding. Fischler in 1910–11 (14) noted the acute degeneration in the liver and fat necrosis following Eck fistula such as could be produced by pancreatic injury. These could be prevented by preliminary injections of trypsin. The meat intoxication, according to his view, was caused by an alkalosis due most probably to ammonia unneutralized by the liver and this condition could be relieved by acids. Michaud in 1911 (15) showed that Eck fistula prevented adrenal glycosuria but that when such an animal is fed sugar by mouth, the blood sugar either remains normal or increases within normal limits, as in any intact animal. In 1911 Voegtlin and Bernheim (16) showed that the bile pigments and bile acids were decreased in an Eck fistula dog to such an extent that the ligation of the common duct was not followed by jaundice and by only a trace of bile in the urine. They noted also the occasional attacks of convulsions, somewhat similar to those occurring in tetany, produced by meat feeding and showed that these could be prevented by feeding plenty of calcium in the form of bone. They inferred that the Eck fistula condition was compatible

with life if care was used in feeding. In 1911 Towles and Voegtlin (17) showed that there was no essential difference in the metabolism of creatin and creatinin in a normal and an Eck fistula dog and concluded that the liver is not an organ of prime importance in the metabolism of these substances and that the creatinin could hardly be held accountable for the toxic symptoms evoked on meat feeding. In thus excluding the portal circulation from the liver and diverting the portal stream directly into the systemic venous circulation, there is seen to be a reduction in the liver function: *a*, in the synthesis of the urea from ammonia salts; *b*, in the production of bile salts and bile acids; and *c*, in the lowered carbohydrate tolerance. It is with this latter feature that this paper is mainly concerned.

Conduct of experiments. Normal healthy dogs were used throughout the experiment—good-sized animals and those only who proved adaptable to sugar feeding on the pre-operative tolerance determinations. To have comparable results, weighed quantities from the same lot of sugar were used on all the animals. Sugar feedings were under as uniform conditions as possible,—namely, one sugar feeding a day, on empty stomach, 4 to 5 hours after meals, with definite amounts of water for dissolving the sugar and avoidance of all food or drugs with the feedings. Sugar in the urine was tested for and determined by Fehling, Nylander, fermentation and the polariscopic methods. The animals were fed on a mixed diet of boiled meat and bread, and were kept in cages during the tests. The urine was collected from beneath the cages.

The Eck fistula operation consisted of making a typical lateral anastomosis between the portal vein and the inferior vena cava using small enterostomy clamps (Jerusalem-Jeger) and silk for the sutures. On completion of the anastomosis, the portal vein was doubly ligated and transected between the ligatures, near the liver hilus.[1] Posterior

[1] Following Eck fistula operation, the animals remained apparently well and in all outward respects normal. There is an early but decreasing tendency to loss of weight and at first bounteous feedings are necessary to maintain the general body weight. When fed on a meat diet for a great length of time or by the addition of meat extract to a diet for several days, convulsive attacks somewhat suggestive of tetany are likely to occur. These are characterized by disorientation, indifference to surroundings, muscular twitchings, paralysis of the limbs, rigidity of back, ataxia and fine tremors. These may last as long as 48 hours and may lead to the death of the animal. The attacks are usually preceded by a period during which the animal seems below par, may be dull and drowsy or may be unusually restless or nervous. At this stage the acute attack may occasionally

lobe hypophysectomy was performed by the intracranial method described by Crowe, Cushing and Homans (18). This consists of contralateral temporal openings through bone and dura which allows the brain to be lifted and dislocated upwards through one decompression opening. The posterior lobe is then enucleated from the dangling gland from below, through the temporal opening of the opposite side.

Protocols

Animal A. Well-nourished, normal male dog, weight 11.2 kilos
April 10–16. Pre-operative cane sugar tolerance, 130–140 grams
April 17–28. Eck fistula operation—uneventful convalescence
April 28–May 15. *Post-operative sugar tolerance. Weight 10.8 kilos*
Cane sugar, 14 grams. Laevo sugar in urine
Glucose, 55–60 grams
Laevulose, 7–8 grams
May 25–June 3. *2nd post-operative sugar tolerance*
Cane sugar, 14–25 grams
Glucose, 55–60 grams
Laevulose, 7–8 grams
June 18–25. *3rd post-operative sugar tolerance. Weight 10.8 kilos*
Cane sugar, 14–15 grams
Glucose, 55–60 grams
Laevulose, 7–8 grams. Mild convulsive attacks
July 7–15. *4th post-operative sugar tolerance. Weight 10.2 kilos*
Cane sugar, 14–15 grams
Glucose, 55–60 grams
Laevulose, 7–8 grams

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be aborted by feeding the animal plenty of bone, a substance for which he seems particularly desirous. Injections of calcium chloride or feedings of calcium lactate do not seem to have any effect when the acute attacks have already begun. After the attack has worn away the animal seems to be in normal condition again. One of the animals died during a severe second attack while the other two dogs had two and five attacks respectively. If care is taken to keep the animal on a mixed diet and excess of meat is avoided and if there is plenty of calcium in the form of bone, there seems to be no reason why an Eck fistula animal should not live for a long period of time. The animals reported here were observed for 139, 340 and 408 days respectively after the Eck fistula operation; the two latter finally succumbed to conditions unassociated with the fistula operation.

On autopsy the only pathological findings of interest are those associated with the liver. This organ is small, hard, firm and rather tougher than normal. The gall bladder and bile ducts appear to be normal. On section, the liver lobules seem to be decidedly smaller than normal with central atrophy and some fatty degeneration, conditions which have been produced experimentally by a reduction of the blood supply of the liver itself.

July 17–30. Hypophysectomy—posterior lobe removal—ether
Urinary output from 1520 cc. to normal in 5 days. No permanent polyuria
Slight trace of sugar in the first specimen; no permanent glycosuria
August 3–10. *1st post-operative tolerance. Weight 11.2 kilos*
Cane sugar, 14–15 grams
Glucose, 45–50 grams
Laevulose, 7–8 grams
August 18–24. *2nd post-operative tolerance*
Cane sugar, 14–15 grams
Glucose, 50–55 grams
Laevulose, 7–8 grams
September 6–13. *3rd post-operative tolerance. Weight 11.3 kilos*
Cane sugar, 14–15 grams
Glucose, 55–60 grams
Laevulose, 7–8 grams
September 20–28. *4th post-operative tolerance*
Cane sugar, 14–15 grams
Glucose, 60–70 grams
Laevulose, 7–8 grams
October 5–13. *5th post-operative tolerance. Weight 10.8 kilos*
Cane sugar, 14–15 grams
Glucose, 65–70 grams
Laevulose, 7–8 grams
October 20–26. *6th post-operative tolerance*
Cane sugar, 14–15 grams
Glucose, 65–70 grams
Laevulose, 7–8 grams
November 6–15. *7th post-operative tolerance*
Cane sugar, 14–15 grams
Glucose, 65–70 grams
Laevulose, 7–8 grams. Mild convulsions
November 24–30. *8th post-operative tolerance. Weight 10.8 kilos*
Cane sugar, 14–15 grams
Glucose, 65–70 grams
Laevulose, 7–8 grams

Summary of animal A

	NORMAL TOLERANCE	AFTER ECK FISTULA	AFTER HYPOPHYSECTOMY
	grams	*grams*	*grams*
Cane sugar	130–140	14–15	14–15
Glucose		55–60	65–70
Laevulose		7–8	7–8

Animal used for pancreatectomy—died May 24 of pneumonia.

In this, the first of the Eck fistula animals in good condition, the saccharose tests even in small amounts gave sugar which proved to be laevo-rotatory. As the saccharose is necessarily broken up into dextrose and laevulose in the process of its absorption and utilization in the body, it seemed necessary to resort to separate tests for the tolerance of the two sugars before the operation. It showed also that the assimilation tests under the circumstances with a complex sugar like saccharose, such as had been used, was capable of misinterpretation; for with hepatic insufficiency there would always be a low assimilation limit and a laevo-rotatory substance in the urine whereas the glucose tolerance itself might be little modified.

Animal B. Well-nourished, normal male dog, weight 13.3 kilos

May 11–June 6. *Pre-operative sugar tolerance. Weight 13.3 kilos*
Cane sugar, 170–175 grams
Glucose, 110–115 grams
Laevulose, 85–90 grams

June 6–19. Eck fistula operation—uneventful convalescence

June 19–July 10. *Post-operative sugar tolerance*
Cane sugar, 10–15 grams
Laevulose, 7–8 grams
Glucose, 75–80 grams.

July 12–22. *Hypophysectomy*—posterior lobe removal—ether. *Weight 12.3 kilos*
Urinary output 1360 cc. to normal in 7 days. No permanent polyuria
No glycosuria

July 23–August 2. *1st post-operative tolerance*
Laevulose, 8–9 grams
Cane sugar, 10–15 grams
Glucose, 70–80 grams

August 10–18. *2nd post-operative tolerance. Weight 12.6 kilos*
Laevulose, 8–9 grams
Cane sugar, 10–15 grams
Glucose, 70–80 grams

August 28–September 8. *3rd post-operative tolerance*
Laevulose, 8–9 grams
Cane sugar, 10–15 grams
Glucose, 100–110 grams

September 17–24. *4th post-operative tolerance. Weight 12.7 kilos*
Laevulose, 8–9 grams. 1st series of convulsions
Cane sugar, 10–15 grams
Glucose, 100–110 grams

October 4–10. *5th post-operative tolerance*
Laevulose, 8–9 grams
Cane sugar, 10–15 grams
Glucose, 100–110 grams

October 17–22. *6th post-operative tolerance. Weight 12.9 kilos*
Laevulose, 8–9 grams
Cane sugar, 10–15 grams
Glucose, 100–110 grams. 2nd convulsive attack
Animal died on October 24—2nd series of convulsions

Summary of animal B

	NORMAL TOLERANCE	AFTER ECK FISTULA	AFTER HYPOPHYSECTOMY
	grams	*grams*	*grams*
Cane sugar	170–175	10–15	10–15
Glucose	110–115	75–80	100–110
Laevulose	85–90	8–9	8–9

Animal C. Well-nourished, normal male dog, weight 14.6 kilos
October 12–November 1. *Pre-operative sugar tolerance*
Cane sugar, 170–175 grams
Glucose, 120 grams
Laevulose, 90 grams. *Weight 16 kilos*
November 2–10. Eck fistula operation—uneventful convalescence
November 10–December 1. *Post-operative sugar tolerance*
Cane sugar, 20–25 grams
Laevulose, 7–8 grams
Glucose, 100 grams. *Weight 12.7 kilos*
December 2–12. *Hypophysectomy*—posterior lobe removal—ether
Urinary output 1970 cc. to normal in 10 days. No permanent polyuria
No glycosuria
December 12–January 1. *1st post-operative tolerance*
Laevulose, 7–8 grams
Glucose, 80–90 grams
Cane sugar, 20–25 grams. *Weight 12.7 kilos*
January 12–19. *2nd post-operative tolerance*
Laevulose, 7–8 grams
Glucose, 80–90 grams
Cane sugar, 20–25 grams. 1st attack of convulsions
January 29–February 6. *3rd post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 80–90 grams. *Weight 12.9 kilos.* 2nd attack of convulsions
February 17–24. *4th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 80–90 grams
March 14–23. *5th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 90–100 grams. *Weight 13 kilos*

April 9–20. *6th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 110–120 grams. 3rd attack of convulsions
May 15–26. *7th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 140–150 grams
June 8–14. *8th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 140–150 grams. *Weight 13.1 kilos*
July 6–14. *9th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 140–150 grams. 4th attack of convulsions
July 28–August 4. *10th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 140–150 grams. *Weight 13.3 kilos*
August 11–16. *11th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 140–150 grams
August 24–30. *12th post-operative tolerance*
Laevulose, 7–8 grams
Cane sugar, 20–25 grams
Glucose, 140–150 grams. *Weight 13.3 kilos*

Summary of animal C

	NORMAL TOLERANCE	AFTER ECK FISTULA	AFTER HYPOPHYSECTOMY
	grams	*grams*	*grams*
Cane sugar	170–175	20–25	20–25
Glucose	120–130	90–100	140–150
Laevulose	90–95	7–8	7–8

Animal used for pancreatectomy—died December 15.

The tolerance of an animal for sugar is the lowest dose of that sugar, following the ingestion of which, sugar just appears in the urine—the threshold dose producing glycosuria. It is dependent upon a number of factors such as the variety of sugar, the general state and condition of the animal, the manner and method of administering the sugar. This threshold dose is an indefinite amount varying between rather wide limits. Of the several methods of introducing sugar,—intra-

venous, subcutaneous and alimentary,—the two former give the lower values and the latter, the most frequently used, the highest values. For alimentary glycosuria the absorption rate from the gastro-intestinal tract must exceed the storage and utilization power of the tissues so that a hyperglycemia is produced with a blood sugar content high enough to overcome the normal impermeability of the kidney. It is evident that anything that concerns the speed of absorption, the utilization rate, the storage power of the tissue and the permeability of the kidney will result in changes in the tolerance. It is also evident that small amounts of sugar, through rapid absorption, will produce an abrupt rise in the blood sugar content with resultant glycosuria while larger amounts with slower absorption may be unaccompanied by the appearance of the sugar in the urine. The rate of absorption of sugar from the gastro-intestinal tract is not a constant factor and, as such, influences markedly the amount of sugar brought to the blood and tissues for utilization. The higher the tolerance, the more inaccurate the determination is and the greater the limits of variance. With the lower tolerance sugars, the determination is less difficult and the limits more readily defined. In many cases it is extremely difficult, and in some animals impossible, to obtain the tolerance with high tolerance sugars on account of the intolerance of stomach and intestinal tract for the huge and abnormal masses required for one feeding. The tolerance is usually stated in grams per kilo of body weight. This is open to grave error as young animals have a much lower tolerance per kilo than adult animals and obesity, as is well known, is not accompanied by a proportionate increase in the sugar tolerance. The indefiniteness of alimentary sugar tolerances is readily seen when one peruses the literature for tolerances determined by different investigators. Hofmeister's (19) tolerances for glucose in dogs ranged from 1.3 to 5.8 grams with 2 to 3 grams per kilo as the average. This is an unusually low tolerance, in fact much lower than that after intravenous administration and is rather out of the question. Pflueger (20) found some 11 to 16 grams per kilo. That the method of administration had a definite effect upon the tolerance is seen by his figures of 11 to 12 grams when the dextrose was given with meat stew and of less than 8 grams per kilo when it was given with soup. Schlessinger (21) found the tolerance to be about 10 to 11 grams per kilo and that small young animals had a lower tolerance per kilo than the larger adult animals. Boeri and De Andreis (22) found values of 4 to 6 grams for fasting animals and 10 to 13 grams for well-nourished animals. Pratt and

Spooner (23) found values of 11.5 grams for dogs. Quarta (24) found an average of 4 grams for male and 10 grams for female dogs. Sex has been found to have no appreciable effect upon the sugar tolerance and his value of 4 grams per kilo is entirely too low. Filippi's values were 8 to 10 grams for fasting animals and he lays great stress upon the fact that to obtain comparable results in alimentary feeding, sugar should be given under certain definite conditions. The tolerance values given for laevulose are few in number. Quarta gave the value as between 3 to 4 grams per kilo and de Filippi as about 1.5 gram. The tolerance for saccharose shows the same wide variation. Hoeppe–Seyler (25) sets it at 20 to 30 grams per kilo, Hofmeister at 3.6 grams and de Filippi at about 4 grams per kilo.

The osmotic irritation, the gastric and intestinal intolerance for large and abnormal masses of sugar, the lack of any control over the varying rates of absorption, make the alimentary method, though practically the only feasible one, far from an ideal one for investigative purposes. With an adaptable animal, under uniform conditions, comparative results on the same animal may however furnish data of some value.

Table 1 gives the tolerances obtained in this investigation. The normal values differ considerably from those given by de Filippi. The saccharose tolerances agree fairly closely with those obtained by Goetsch, Cushing and Jacobson who found in their series of animals weighing 9 to 11 kilos, a tolerance of 9 to 13 grams per kilo of body weight.

Tolerance after Eck fistula operation. Following Eck fistula operation there are marked changes in the carbohydrate tolerance ascertained by alimentary feeding. Subsequent to this surgical procedure there is usually a marked loss in body weight, and some of the post-operative tolerances given in the above table are undoubtedly high since they are calculated upon the lowered body weight. The glucose tolerance is modified to a less degree than the laevulose or cane sugar tolerance. This seems to indicate that the muscle and the body tissue generally can manage very well the storage and utilization of the glucose that is shunted to them by the Eck fistula operation and leads one to infer that the liver is an organ not absolutely necessary for the utilization of that sugar.

The most marked changes occur in the laevulose tolerance. In an Eck fistula animal a small amount of laevulose ingested is followed by a laevulosuria, there being almost an intolerance for that particular sugar. Muscular tissue can undoubtedly utilize a small amount of

laevulose as is evidenced by muscle perfusion experiments. It is questionable whether the muscles can change laevulose into glycogen and to any large extent thus utilize it. It seems quite probable that the liver acts as an organ of storage for the laevulose and alters it in some manner or other to make it more utilizable by the tissues. The liver evidently is indispensable for laevulose metabolism.

TABLE 1

Sugar tolerance; grams per kilogram of body weight

	NORMAL	AFTER ECK FISTULA	AFTER HYPOPHYSECTOMY
		Dog A	
Weight	11.4 kilos	11.4–10.2 kilos	10.8 kilos
Cane sugar	12.0 grams	1.4 grams	1.3 grams
Glucose		5.6 grams	6.2 grams
Laevulose		0.7 gram	0.7 gram
		Dog B	
Weight	13.3 kilos	13.3–12.3 kilos	12.3–12.9 kilos
Cane sugar	13.3 grams	0.9 gram	1.0 gram
Glucose	8.4 grams	5.9 grams	8.0 grams
Laevulose	6.5 grams	0.6 gram	0.6 gram
		Dog C	
Weight	14.6 kilos	16.0–12.5 kilos	12.7–13.3 kilos
Cane sugar	11.7 grams	2.0 grams	1.7 gram*
Glucose	8.2 grams	8.0 grams	11.9 grams
Laevulose	6.2 grams	0.6 grams	0.6 gram

* As this animal had lost considerable weight after the Eck fistula operation these values figured on the body weight are as a consequence rather high.

The tolerance changes shown by saccharose are similar to those shown by laevulose. This sugar is broken down into dextrose and laevulose in the process of assimilation and the split products exert their respective effects upon the tolerance. Small doses of saccharose given to an Eck fistula animal are followed by a laevulosuria.

These changes in tolerance subsequent to Eck fistula operation in the dog, though the absolute tolerance values are quite different, are similar to those reported by de Filippi and are given here as confirmation of his work.

Tolerance changes following subsequent hypophysectomy. Following hypophysectomy in an Eck fistula animal the following changes were noted:

1. Transient glycosuria. In only one of the three cases was there a glycosuria following the operation. This was of a temporary character and appeared only in the first specimen of urine voided. There was no persistent or permanent glycosuria. Such a slight transient glycosuria was also found in three out of ten cases reported by Goetsch, Cushing and Jacobson, in their series of posterior lobe hypophysectomy. It is questionable whether this slight occasional glycosuria is due to the removal of the gland or incidental to the operation itself.

2. Polyuria. A definite post-operative polyuria was noted in all the three cases. Following operation there was a high urinary output of 1560, 1360 and 1970 cc. per day, gradually decreasing in amount so that the normal pre-operative urinary output was reached in 5 to 7 and 8 days respectively. The fluid intake on these days was increased also in proportionate amounts. There was no permanent or persistent polyuria as the result of the posterior lobe removal.

3. Increased carbohydrate tolerance. There was a moderate but definite increase in the tolerance for glucose after posterior lobe removal in all of the three animals, while the cane sugar and laevulose values remained essentially unmodified at their low levels. This augmentation of glucose tolerance came on extremely late, however, when compared to the augmentation following hypophysectomy in normal animals. In the series of hypophysectomies described by Cushing, Goetsch and Jacobson there was a transient lowering of carbohydrate tolerance followed by augmentation of tolerance reaching its height or greatest value from 9 to 21 days after the posterior lobe removal. In animal A there is a post-operative lowering of tolerance for 51 days, augmentation of tolerance during 28 days, making 79 days in all before the high tolerance was reached. No change in tolerance was noted subsequently during 51 days of observation. In animal B there was no definite post-operative lowering of tolerance, the tolerance reaching its highest value 58 days after operation with no change for 44 days subsequent observation. Animal C showed a low post-operative tolerance for 111 days, augmentation during 84 days or 195 days before the high value was reached. There was no change in tolerance on 96 days of further observation. The conclusion is quite evident that this augmented tolerance, indicative of increased storage and utilization capacity for converted carbohydrates, is the result of some extra-hepatic

function. It is most probably associated with the glycogenic and glycogenolytic power of the muscle tissue itself. Whatever function or capacity the liver has in this direction is readily assumed by the muscles themselves when the liver is removed from the portal circulation.

CONCLUSIONS

1. Eck fistula animals have an extremely low tolerance for laevulose. The liver is evidently essential for laevulose metabolism. The function of converting laevulose into glycogen is possessed by the liver; this function is permanently lost when the portal blood is diverted into the vena cava by Eck fistula.

2. Glucose tolerance is only slightly modified in Eck fistula animals. The liver is not entirely essential for glucose metabolism. The muscles undoubtedly perform well the functions of glycogenesis and glycogenolysis when the liver is shunted out of the portal circulation.

3. The glycogenic capacity of muscle is increased following posterior lobe removal in an Eck fistula animal as in an intact animal. The augmentation of tolerance is, however, considerably slower. No augmentation of laevulose tolerance is noted.

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THE GASTRIC RESPONSE TO FOODS[1]

XII. The Response of the Human Stomach to Pies, Cakes and Puddings

RAYMOND J. MILLER, HARRY L. FOWLER, OLAF BERGEIM, MARTIN E. REHFUSS and PHILIP B. HAWK

From the Laboratory of Physiological Chemistry of Jefferson Medical College, Philadelphia

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Among the preparations classed under the heads of pies, cakes and puddings are a great variety of food products, the majority of which are commonly used as desserts although, in this era of quick lunches, pies, doughnuts, cinnamon buns, etc., frequently usurp the position in the diet of more substantial foods. For this misuse there can only be the excuse of necessity. However, the pies, cakes and puddings include many highly nutritious and most pleasing foods for whose proper preparation the capable cook may well be praised.

In spite of their very wide use there is a general impression that pies, cakes and puddings as a class are rather indigestible. The blame for this may rest in part on the diversity of cooking, in part on the depressing effect on the appetite of these foods if very sweet and eaten between meals, and in part to their being frequently crammed into an already well-filled stomach.

Little study has been made of the response of the stomach to these classes of foods. Some experiments were made by Beaumont (1), although amounts were not controlled. This author found in his subject that rice left the stomach in 1 hour; sago, in 1¾ hour; tapioca, in 2 hours; gelatin, in 2½ hours; sponge cake, in 2½ hours, and apple dumpling in 3 hours. Direct comparison with our results cannot be made; but, as an instance, where Beaumont found rice to leave in less than half the time required for gelatin we found the gelatin to leave sooner than any other pudding. Our average time for cake was also distinctly longer than found by Beaumont.

[1] The expense of this investigation was defrayed by contributions from Mrs. M. H. Henderson, The Curtis Publishing Company and Dr. L. M. Halsey.

Our experiments on gastric response were carried out according to a procedure previously described (2). The subjects of the test were normal medical students who were given the food preparations in lieu of breakfast about 8 o'clock in the morning. Residuums were not removed. About 90 experiments were carried out. The acid responses and evacuation times of 81 of these are charted in figures 1 to 28 and summarized in tables 1, 2 and 3.

RESPONSE OF THE HUMAN STOMACH TO PIES

Fruit pies. Among the fruit pies studied were apple, peach, cherry, pumpkin and raisin pies. Apple pie was tried out on four subjects (see figs. 1, 3, 4 and 6). This pie was evacuated in from 2 hours to $2\frac{3}{4}$ hours, or an average emptying time of $2\frac{1}{2}$ hours. Fairly high acidities were developed within an hour to an hour and a half, the highest value attained varying from 83 to 102, with an average of 94. Further, most of this acidity was due to free HCl, showing that these pies have but a slight combining power for acid, and that in spite of this high free acidity gastric evacuation was not delayed.

Peach pie was given to one subject (see fig. 1) who had previously received apple pie. The acid curves for the two pies were practically identical, an acidity of 98 being developed in an hour and a half with peach pie. This latter pie, however, left the stomach half an hour sooner than apple pie.

Cherry pie was tried out in two cases (see figs. 7 and 8). High acidities, due mainly to free HCl, were developed in both cases. The evacuation time of one subject was 3 hours as compared with $2\frac{3}{4}$ hours for apple pie. Not quite so high an acidity was developed on cherry pie, perhaps because its higher sugar content depressed secretion slightly, an effect we have shown sugar solutions to produce (3).

Raisin pie was given to one subject (see fig. 10). It was evacuated quite rapidly (in $2\frac{1}{4}$ hours) or in the same time as required for rhubarb pie, and more rapidly than mince or pumpkin pie. A moderate acidity (with little combined acid) was developed, as might be expected from a pie fairly high in sugar but containing little protein.

Rhubarb pie in general character and gastric response may be classed with the fruit pies. Two subjects were given this pie (see figs. 9 and 11). It was one of the most readily evacuated pies, leaving the stomach in the same time as raisin pie ($2\frac{1}{4}$ hours) and more rapidly than cherry pie. The acid responses were high, averaging 101 at the maximum, nearly all of this being due to free HCl.

TABLE 1

The response of the human stomach to pies

NUMBER	KIND OF PREPARATION	TYPE OF INDIVIDUAL	EVACUATION TIME	HIGHEST TOTAL ACIDITY
1–Son	Pumpkin	Rapid	2:45	99.5
2–Wil	Pumpkin	Slow	2:00	102.5
3–Son	Mince	Rapid	2:45	107.5
4–Wil	Mince	Slow	3:15	105.0
5–Son	Lemon meringue	Rapid	2:30	74.0
6–Wil	Lemon meringue	Slow	2:30	95.0
7–Son	Raisin	Rapid	2:15	94.5
8–Rud	Cocoanut custard	Rapid	2:15	107.0
9–Rud	Custard pie	Rapid	2:30	84.5
10–Rud	Cherry	Rapid	2:45	99.0
11–Wil	Cherry	Slow	3:00	95.0
12–Mil	Peach	Rapid	2:00	98.5
13–Kar	Apple	Rapid	2:00	97.0
14–Lea	Apple	Rapid	2:30	83.0
15–Mil	Apple	Rapid	2:30	95.0
16–Wil	Apple	Slow	2:45	102.5
17–Rud	Rhubarb	Rapid	2:00	106.0
18–Son	Rhubarb	Rapid	2:15	95.0
Average			2:27	90.0
	Pie combinations			
19–Rud	Cherry (with ice cream)	Rapid	3:15	115.0
20–Lea	Apple (with ice cream)	Rapid	2:30	69.5
21–Wil	Apple (with cheese)	Slow	3:00	113.5
Average			2:55	99.0
	Pie crust vs. insides			
22–Lea	Pie crust	Rapid	2:45	66.5
23–Mil	Pie crust	Rapid	2:45	92.5
24–Wil	Pie crust	Slow	3:15	102.5
25–Son	Pie crust	Rapid	2:45	98.5
26–Far	Insides (apple)	Slow	3:15	93.0
27–Mil	Insides (peach)	Rapid	1:30	103.5
28–Rud	Insides (rhubarb)	Rapid	2:15	118.0
29–Wil	Insides (apple)	Slow	2:45	99.0
Pie crust, average			2:52	90.0
Insides, average			2:26	103.0

Pumpkin pie differs somewhat in character from the fruit pies mentioned, but the acid response was not distinctly different. The acid combining power was slightly greater for pumpkin pie. A subject of

TABLE 2

The response of the human stomach to cakes

NUMBER	KIND OF PREPARATION	TYPE OF INDIVIDUAL	EVACUATION TIME	HIGHEST TOTAL ACIDITY
1–Far	Angels' food	Slow	4:30	97.5
2–Mur	Angels' food	Rapid	3:45	100.0
3–Owe	Angels' food	Rapid	2:45	99.0
4–Far	Devils' food	Slow	4:00	92.0
5–Mur	Devils' food	Rapid	3:00	83.0
6–Owe	Devils' food	Rapid	2:45	73.5
7–Gol	Fruit cake, fresh	Rapid	3:15	73.0
8–Mur	Fruit cake, fresh	Rapid	3:15	80.5
9–Gol	Fruit cake, old	Rapid	3:30	78.0
10–Mur	Fruit cake, old	Rapid	3:30	77.5
11–Mur	Lady fingers	Rapid	3:15	75.5
12–Cor	Crullers	Slow	4:15	88.0
13–Lot	Crullers	Rapid	2:15	91.5
14–Lot	Doughnuts	Rapid	2:30	83.0
15–Spe	Doughnuts	Slow	3:00	106.0
16–Cor	Cream puffs	Slow	2:30	107.0
17–Far	Ginger snaps	Slow	3:30	101.5
18–Gol	Spiced cookies	Rapid	2:15	89.0
19–Lot	Spiced cookies	Rapid	2:15	104.0
20–Owe	Chocolate layer cake	Rapid	2:30	26.5
21–Far	Mary Ann cookies	Slow	2:45	70.5
22–Mur	Cinnamon bun	Rapid	2:30	72.0
23–Spe	Cinnamon bun	Slow	2:45	127.5
24–Owe	Ginger bread	Rapid	2:15	65.0
25–Rud	Ginger bread	Rapid	3:00	106.0
26–Rud	Short cake, strawberry	Rapid	3:00	124.5
27–Ree	Short cake, strawberry	Slow	3:15	130.0
28–Spe	Bread with peanut butter	Slow	3:15	120.5
29–Owe	Bread with corn syrup	Rapid	2:15	55.0
Average			3:02	90.0

the slow emptying type required $2\frac{3}{4}$ hours as compared with $2\frac{1}{4}$ hours for raisin and rhubarb pies (see fig. 10). A subject of the rapid emptying type (see fig. 7) required only 2 hours for pumpkin pie as compared with 3 hours for cherry and $2\frac{3}{4}$ hours for apple pie.

Mince, custard and lemon méringue pies. The mince, custard and méringue pies are higher in protein than simple fruit pies and might, therefore, be expected to undergo greater change in the stomach.

Mince pies are generally considered to be the least easily digested of pies, due to their content of meat and spices. This is borne out by our experiments (see figs. 7 and 10). Mince pie required $3\frac{1}{4}$ hours to leave

TABLE 3

The response of the human stomach to puddings

NUMBER	KIND OF PREPARATION	TYPE OF INDIVIDUAL	EVACUATION TIME	HIGHEST TOTAL ACIDITY
1–Dal	Indian	Rapid	2:00	113.5
2–Gle	Corn starch	Slow	2:30	92.5
3–Dal	Chocolate	Rapid	2:00	110.0
4–Spe	Chocolate	Slow	2:00	91.0
5–Dal	Brown Betty	Rapid	2:15	69.5
6–Spe	Brown Betty	Slow	2:45	110.0
7–Kar	Cabinet	Rapid	2:00	87.0
8–Spe	Cabinet	Slow	2:45	82.0
9–Lea	Rice	Rapid	2:00	67.0
10–Mil	Rice	Rapid	2:00	101.0
11–Lea	Rice with raisins	Rapid	1:45	76.5
12–Mil	Cup custard	Rapid	2:00	107.0
13–Gle	Cup custard	Slow	4:15	112.0
14–Dal	Plum	Rapid	2:15	113.0
15–Gle	Plum	Slow	4:30	96.5
16–Kar	Bread	Rapid	2:15	79.5
17–Dal	Bread	Rapid	2:00	108.0
18–Gol	Bread	Rapid	1:30	96.0
19–Kar	Bread	Rapid	1:45	104.0
20–McD	Tapioca	Slow	2:30	74.0
21–McD	Apple tapioca	Slow	2:15	106.5
22–Col	Gelatine	Rapid	2:00	61.5
23–Kar	Gelatine	Rapid	1:30	62.0
Average			2:18	92.0

the stomach of an individual who required $2\frac{3}{4}$ hours for apple pie and 3 hours for cherry pie. The other subject required $2\frac{3}{4}$ hours for mince and $2\frac{1}{4}$ hours for either raisin or rhubarb pie. The highest acid response was given with mince pie, and the acid combining power was also markedly greater than for simple fruit pies.

One experiment each was made on plain custard and cocoanut custard pie, the same subject receiving both (see fig. 8). The plain custard

required 15 minutes longer to digest and developed a higher acidity due probably to the slightly higher protein content. The high combined acidities of both would be expected from their known content of milk and egg proteins. The custards required a longer digestion than rhubarb pie but were by no means handled with difficulty inasmuch as cherry pie took 15 minutes longer than either.

Related in composition to the custards are the lemon méringues which, when properly made, are fairly high in eggs. They leave the stomach in about the same time as the custards ($2\frac{1}{2}$ hours) and show a less rapid development of acidity than other pies (figs. 7 and 10).

COMPARATIVE RESPONSE OF THE STOMACH TO PIE CRUST, CONTENTS AND WHOLE PIES

The popular opinion that pie crusts are the least digestible portions of ordinary pies would also appear probable from their known compositions, possessing as they do most of the protein and fat of many pies, especially fruit pies. The absolute digestibility of pie crusts must, of course, depend a good deal on their texture and composition. The response of the stomach to pie contents would be expected to approximate that of fruit sauces or puddings of similar composition. Thus we have found (4) that fruits in general leave the stomach very rapidly with the development of considerable free but little combined acidity.

Pie crust alone was given in 100-gram portions to four subjects (figs. 2, 5, 6 and 11). This was evacuated in from $2\frac{3}{4}$ to $3\frac{1}{4}$ hours. In the case of one subject the contents of a peach pie left the stomach in $1\frac{1}{2}$ hour as compared with $2\frac{3}{4}$ hours for the crust. For apple pie and crust the required periods were $2\frac{3}{4}$ and $3\frac{1}{4}$ hours respectively. Pie crust thus remains in the stomach distinctly longer than an equal weight of the contents of apple or peach pie. The acid responses of the two were not so different, although crust usually gave a slower rise with a more sustained curve.

The whole pie naturally is intermediate between pie crust and contents in the response it evokes. Thus subject "Mil" (figs. 1 and 2) retained peach pie 2 hours and contents $1\frac{1}{2}$ hour. Another subject, "Rud" (fig. 9) required a few minutes longer for the rhubarb alone than for the pie, while a third subject, "Wil," took $2\frac{3}{4}$ hours in each case (fig. 6). Thus these fruit pies require little longer to digest in the stomach than the interiors alone.

If crust is compared with whole pie, we find in the case of "Mil" the pies requiring 2 to 2½ hours and crust 2¾ hours; in the case of "Son" 2¼ hours for rhubarb pie and 2¾ hours for crust alone. Subject "Wil" required 2½ to 3¼ hours for pies (mince being the slowest) and 3¼ hours for crust. Crust thus takes somewhat longer to digest in the stomach than the contents of all but the least digestible pies.

It will be seen, therefore, that the response of the stomach to whole pies approximates that of the pie contents, in spite of the fact that the crust alone requires a distinctly longer digestion period. We did not, however, find that crust was in any sense indigestible, although 100 grams of it were given at a time.

PIE WITH ICE CREAM AND WITH CHEESE

To the usual 100-gram portions of apple and cherry pie were added in each of two cases 50 grams of vanilla ice cream. The responses are charted in figures 4, 5 and 8. The cherry pie alone required 2¼ hours. With ice cream it remained in the stomach half an hour longer. Apple pie, however, showed the same evacuation time (2½ hours) in each case. In one case the total acidity was slightly higher after adding ice cream; in the other the reverse was true. It is clear, therefore, that ice cream, though greatly increasing the food value of the dish, need not necessarily increase the burden of the stomach to any marked extent.

Twenty grams of cream cheese were added to apple pie in one case and compared with apple pie alone (see fig. 6). It will be noted that the acid responses in the two cases do not differ greatly, although naturally cheese increases the acid combining power of the food ingested. The addition of the cheese delayed evacuation about a quarter of an hour, and led to a slight secretion of gastric juice after the stomach was otherwise empty.

THE RESPONSE OF THE HUMAN STOMACH TO CAKES

Among the cakes and related products tested out by us were angels' food, devils' food, fruit cake, chocolate layer cake, lady fingers, strawberry short cake, crullers, doughnuts, cream puffs, cinnamon buns, ginger bread and cookies of different kinds. One hundred grams were given in each case.

Angels' food, devils' food, fruit cake and short cake. Angels' food was given to three men. A few days later an equal amount of devils' food was given to each of the same three subjects for comparison (see figs. 12, 13 and 15). It was found that angels' food cake remained on the average 20 minutes longer in the stomach than devils' food, the evacuation time of these cakes ranging from $2\frac{3}{4}$ to $4\frac{1}{2}$ hours. This may well be related to the greater protein content of the angels' food which is particularly high in eggs. The distinctly higher acidities developed in the stomach in the case of angels' food cake, and the much more pronounced acid combining power must also be due to the high protein content of this cake as compared with devils' food which, however, is higher in calories due to its sugar and chocolate content.

Chocolate layer cake (fig. 15) left the stomach of a subject of the rapid-emptying type in $2\frac{1}{2}$ hours or a few minutes sooner than devils' food cake. The acidity developed was low, due perhaps in part to the depressing action of the sugar icing on secretion.

Old and fresh fruit cake were compared, using two subjects on each (see figs. 13, 14 and 17). It is a popular belief that old fruit cake is much more easily digested than the same cake in the fresh condition. Portions of freshly baked cake were given to the men, and three weeks later the same subjects were given other portions of the cake which had been kept in tin at room temperature for that period.

In each case it was found that while the fresh cake required $3\frac{1}{4}$ hours for gastric digestion the older cake required $3\frac{1}{2}$ hours. The acid responses were practically identical on the fresh and older cake. No distinct difference in gastric response to fresh and old fruit cake could, therefore, be detected.

Two subjects were given strawberry shortcake (75 grams cake and 25 grams of berries) without cream. This cake left the stomach in moderate time (3 to $3\frac{1}{4}$ hours) and gave rise to the development of high intragastric acidities, in part probably due to the acidity of the berries themselves.

Lady fingers were given to one subject (see fig. 13). The evacuation time of these was $3\frac{1}{4}$ hours as compared with 3 hours for devils' food and $3\frac{1}{4}$ hours for fresh fruit cake.

Doughnuts, crullers, ginger bread, cinnamon buns and cream puffs. Ginger bread was given to two subjects (see figs. 9 and 16). It remained in the stomach from $2\frac{1}{4}$ to 3 hours or a few minutes less than strawberry shortcake or chocolate layer cake and a shorter time than devils' food or angels' food cake. Ginger bread brought about a mod-

erate stimulation of acid secretion with, in one case, a rather slow development. Evidently the ginger did not induce any increased secretion of gastric juice.

Cinnamon buns were tested out on two subjects (figs. 14 and 21), and required from $2\frac{1}{2}$ to $2\frac{3}{4}$ hours. In one subject these buns left the stomach sooner than fruit cake, angels' or devils' food cake or lady fingers. The other subject evacuated cinnamon buns a few minutes sooner than doughnuts. In acid response the buns were found to follow the general trend of the cakes just mentioned, not attaining, however, to the level of angels' food cake. Neither is the acid response as high as that of bread with peanut butter. Probably the sugar of the buns depresses secretion somewhat.

Bread with peanut butter (100 grams bread and 25 grams of peanut butter) remained in the stomach $3\frac{1}{4}$ hours as compared with $2\frac{3}{4}$ hours for cinnamon buns. The acidity developed was considerably higher in the case of bread with peanut butter (see fig. 22).

Bread with corn syrup (80 grams of bread and 20 grams corn syrup) left the stomach in $2\frac{1}{4}$ hours (fig. 16) or in the same time as ginger bread and a little sooner than chocolate layer cake or devils' food. The acidity developed was only moderate, being slightly lower than for ginger bread, the syrup probably depressing secretion.

Cream puffs were given to one subject (fig. 19). They developed an acidity of over 100 and left in $2\frac{1}{2}$ hours or much sooner than crullers.

Crullers and doughnuts were compared on one subject (fig. 20). The doughnuts remained in the stomach 15 minutes longer than the crullers or $2\frac{1}{2}$ hours. The maximum acidities developed were about the same in each case, but the acid development was slower in the case of crullers due, perhaps, to their higher sugar and fat content. One subject required $4\frac{1}{4}$ hours for crullers. His digestion did not, however, appear to be entirely normal on the day of this test. A third subject required for doughnuts a digestion time of 3 hours as compared with $2\frac{3}{4}$ hours for cinnamon buns (see fig. 21). It seems, therefore, that doughnuts are handled a little less readily than crullers, but that neither of these are unusually difficult for the normal stomach to handle.

The response of the stomach to cookies. Spiced cookies, ginger snaps and Mary Ann cookies were studied in a few cases. The Mary Ann cookies remained in the stomach of a subject of the slow-emptying type for $2\frac{3}{4}$ hours as compared with 4 hours for devils' food and $4\frac{1}{2}$ hours for angels' food cake, and this in spite of the higher content of the cookies in dry matter. Ginger snaps required in this subject $\frac{3}{4}$ hour

longer than the Mary Anns. A high acidity was developed on ginger snaps, but this was very slow in development, indicating that the ginger depressed gastric secretion.

Spiced cookies left the stomach in 2¼ hours in both cases where they were tried out. They remained the same time as crullers but not so long as doughnuts. They left an hour sooner than fruit cake. Fairly high acidities were developed by these cookies.

It appears, therefore, that cookies, in spite of their high content of dry matter, leave the stomach sooner than many cakes, and in really equivalent amounts would be handled more readily by the stomach. This may in part be due to their more granular texture.

THE DIGESTION OF PUDDINGS IN THE HUMAN STOMACH

The following puddings were studied: vanilla corn starch, chocolate corn starch, tapioca, apple tapioca, gelatin, Indian pudding, bread pudding, Brown Betty, cabinet, rice, rice with raisins, cup custard and plum pudding. One hundred grams of pudding were given in each case.

Starch and tapioca puddings. Five experiments were carried out on corn starch and tapioca puddings (see figs. 22, 23, 24 and 26). Vanilla and chocolate corn starch and plain tapioca and apple tapioca were tested. All of these puddings left the stomach in from 2 to 2½ hours. Apple tapioca was evacuated 15 minutes sooner than the plain pudding. It developed a higher acidity with less combined hydrochloric acid. The starch puddings left the stomach rather quickly and developed a moderately high acidity (91 to 110).

Cereal puddings, cabinet, Brown Betty, bread pudding, rice pudding and Indian pudding. Bread pudding quickly developed in the stomach a high total and moderate combined acidity and was rapidly digested, leaving the stomach in from 1½ to 2 hours in the case of subjects of the rapid-emptying type (see figs. 17, 24 and 27). Brown Betty pudding remained in the stomach 2¼ hours as compared with 2 hours for bread pudding. A subject of the slow-emptying type required 2¼ hours for this pudding (figs. 22 and 24).

Cabinet pudding left the stomach of a subject of the rapid type in 2 hours as compared with 1¾ hours for bread pudding (see fig. 27). A subject of the slow type (see fig. 22) required 2¾ hours or the same time as for Brown Betty. Cabinet developed a lower acidity than bread pudding.

Indian pudding gave a response practically identical with that of bread pudding, leaving the stomach in 2 hours and developing a high acid point of 113 at an hour and a half, the high protein content of this pudding being undoubtedly responsible for the high combined acidities found (see fig. 25).

Rice pudding was tried both with and without raisins (see figs. 1 and 4). The acid responses were in each case practically the same. The evacuation times on plain rice pudding were in each case 2 hours. Rice pudding with raisins required in one case 2 hours and in the other only $1\frac{3}{4}$ hours. The combined acidities were practically the same in each case. The rice puddings are thus among the more easily digested puddings.

Gelatin and plum puddings and cup custards. A widely used gelatin dessert of strawberry flavor was given to each of two men (see figs. 27 and 28). It left their stomachs very rapidly (in $1\frac{1}{2}$ to 2 hours) and gave rise to very little stimulation of acid secretion. The low combined acidities also indicate that this product leaves the stomach too soon and with too little stimulatory effect to be markedly altered in the stomach or to throw any considerable burden upon it.

Plum pudding was found to be one of the less readily digested puddings (see figs. 23 and 27). Its relatively high food value must, however, be borne in mind. A subject of the rapid-emptying type required $2\frac{1}{4}$ hours to evacuate this food as compared with $1\frac{1}{2}$ hours for gelatin and $1\frac{3}{4}$ hours for bread pudding. A subject of slow-emptying type required $4\frac{1}{2}$ hours for plum as compared with $2\frac{1}{2}$ hours for corn starch pudding. Moderately high total and combined acidities were developed.

Cup custards remained in the stomach about the same time as Brown Betty pudding and a few minutes longer than bread pudding, corn starch or Indian puddings, but not so long as plum pudding (see figs. 23 and 24). Being very high in protein, custards would be expected to show a rapid gastric stimulation with much combined acidity. This was found to be the case.

COMPARATIVE RESPONSES OF THE STOMACH TO PIES, CAKES AND PUDDINGS

From tables 1, 2 and 3 the average acid responses and evacuation times of all subjects on pies, cakes and puddings may be obtained. They show that the grand average evacuation times were for puddings,

pies and cakes, 2:18, 2:27 and 3:02, respectively. The average highest total acidity was practically 90 in each of the three cases.

Perhaps a more significant comparison may be obtained from table 4, in which are summarized the results from six subjects, each of whom besides receiving a number of pies or cakes or puddings was also given certain foods belonging to one of the other two classes. It will be seen that here also pies left the stomach sooner than cakes, and puddings still more rapidly.

TABLE 4

SUBJECT	EVACUATION TIME AND ACIDITIES					
	Puddings		Pies		Cakes	
Gol	1:30	96.0			3:00	80.2
Spe	2:30	94.3			3:00	118.0
Kar	1:52	83.1	2:00	97.0		
Lea	1:67	71.5	2:30	76.0		
Mil	2:00	104.0	2:23	100.5		
Rud			2:41	101.4	3:00	115.0
Average	2:00	89.8	2:24	93.7	3:00	104.4

SUMMARY AND CONCLUSIONS

A study was made of the acid responses and evacuation times of nearly fifty pies, cakes and puddings in the normal human stomach. The average evacuation time on puddings for all subjects was 2 hours and 18 minutes as against 2 hours and 27 minutes for pies and 3 hours and 2 minutes for cakes. Averaging the highest total acidities values were obtained for puddings of 92, for pies of 90 and for cakes of 90. Direct comparisons of the three types of foods on the same individuals indicated also that pies were handled more readily than cakes, and pudding somewhat more readily than either.

Fruit pies, such as apple, pumpkin, raisin and peach, left the stomach in from 2 to $2\frac{3}{4}$ hours and developed a moderately high acidity (90 to 100). Most of the acidity is due to free HCl, the acid combining powers of these pies being low. Cherry pie, high in sugar, remained in the stomach a few minutes longer than the above. Rhubarb pie was treated in the same way as fruit pies, leaving the stomach in from 2 to $2\frac{1}{4}$ hours.

Custard pies left the stomach in moderate time ($2\frac{1}{4}$ to $2\frac{1}{2}$ hours) and possessed a fairly high acid combining power. Lemon méringue

showed a similar response except that the acidity developed more slowly. Mince pies required a rather long time to leave the stomach ($2\frac{3}{4}$ to $3\frac{1}{4}$ hours) and developed high total and combined acidities.

Pie crusts alone remained in the stomach distinctly longer than most whole pies or the contents alone of such pies, and gave a lower but more sustained acid curve. On the other hand, differences in evacuation time of whole pies and the contents of such pies were usually very slight so that pies with crust, if properly made, could by no means be classified as difficult for the stomach to handle.

The addition of 50 grams of ice cream to a small piece of pie did not increase the burden of the stomach to any marked extent. The addition of 20 grams of cheese to apple pie increased the digestion time only a few minutes.

Angels' food cake remained distinctly longer in the stomach than devils' food cake and developed a higher total and combined acidity.

Chocolate layer cake left the stomach in moderate time, acid secretion being depressed by the sugar of this cake.

Fresh and old fruit cakes showed almost identical acid responses and evacuation times in the human stomach.

Strawberry shortcakes left the stomach in moderate time (3 to $3\frac{1}{4}$ hours) and developed high intragastric acidities.

Lady fingers left the stomach in 3 hours or in about the same time as other cakes.

Ginger bread evoked a rather slow acid response and left the stomach in moderate time.

Cinnamon buns left the stomach sooner than most cakes but with a similar acid response.

Bread with peanut butter remained in the stomach longer than cinnamon buns and developed a higher acidity.

Bread with corn syrup left the stomach in $2\frac{1}{4}$ hours, the syrup depressing secretion somewhat.

Doughnuts remained in the stomach a few minutes longer than crullers. Acidities developed a little more slowly in the case of crullers, due perhaps to the sugar and fat content. These fried cakes required a digestion time but little longer than the average for cakes.

Cookies, in spite of their high content of dry matter, were found to leave the stomach sooner than most cakes, probably on account of their granular texture. Ginger cookies required a little longer than those less highly flavored, and the acid development was less rapid.

Chocolate corn starch pudding, rice pudding with or without raisins, Indian pudding, bread pudding and gelatin left the stomachs of individuals of the rapid-emptying type very quickly (in $1\frac{1}{2}$ to 2 hours). Rice pudding with raisins left a little sooner than plain rice pudding.

Cabinet pudding, Brown Betty pudding, cup custard and apple tapioca required only a few minutes longer. Plain tapioca remained in the stomach a little longer than apple tapioca. Plum pudding left the stomach slowly.

The highest total and combined acidities were caused by Indian pudding, bread pudding and cup custard, all of these being high in protein. The other puddings developed moderate acidities except gelatin, which produced little acid stimulation and left the stomach very quickly.

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(2) Fishback, Smith, Bergeim, Lichtenthaeler, Rehfuss and Hawk: This Journal, 1919, xlix, 174.
(3) Miller, Bergeim and Hawk: Unpublished data.
(4) Smith, Fishback, Bergeim and Hawk: Unpublished data.

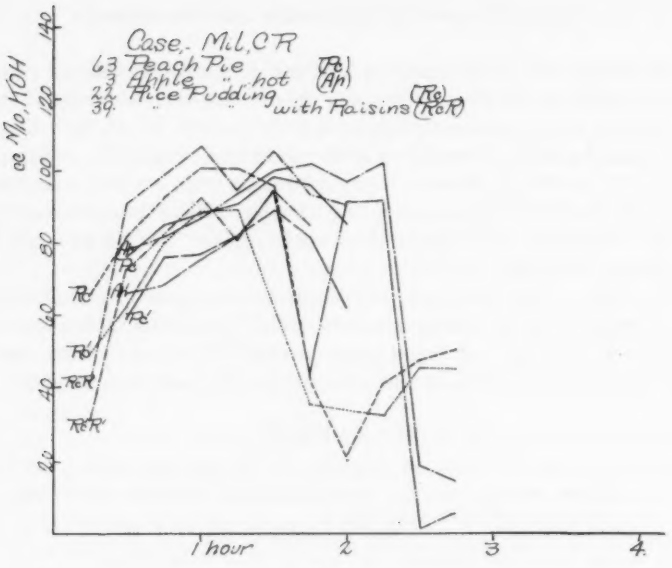
Case. Mil, CR
63 Peach Pie (Pe)
3 Apple " hot (Ap)
22 Rice Pudding with Raisins (Re)(ReR)
39.
cc Mo. KOH
140
120
100
80
60
40
20
Re
Ap
Pe
Pe'
Re'
ReR
ReR'
1 hour
2
3
4

FIG. 1

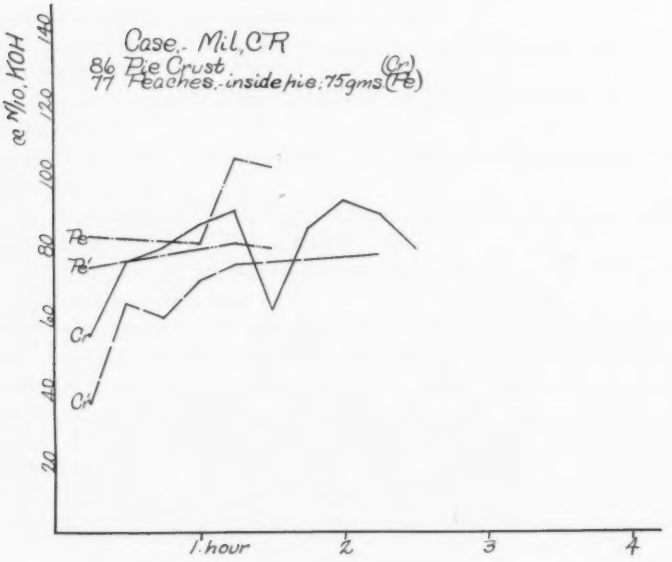
Case. Mil, CR
86 Pie Crust (Cr)
77 Peaches. inside pie. 75 gms (Pe)
cc Mo. KOH
140
120
100
80
60
40
20
Pe
Pe'
Cr
Cr'
1 hour
2
3
4

FIG. 2

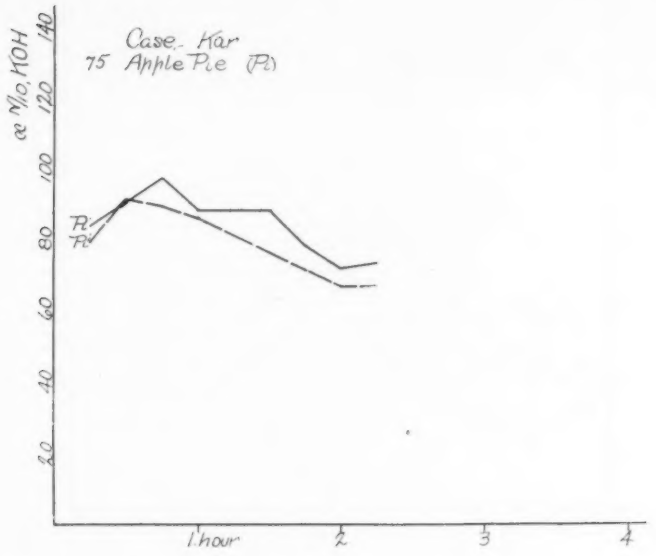
Case. Har
75 Apple Pie (P1)
cc N/10 KOH
140
120
100
80
60
40
20
P1
P1'
1 hour
2
3
4

FIG. 3

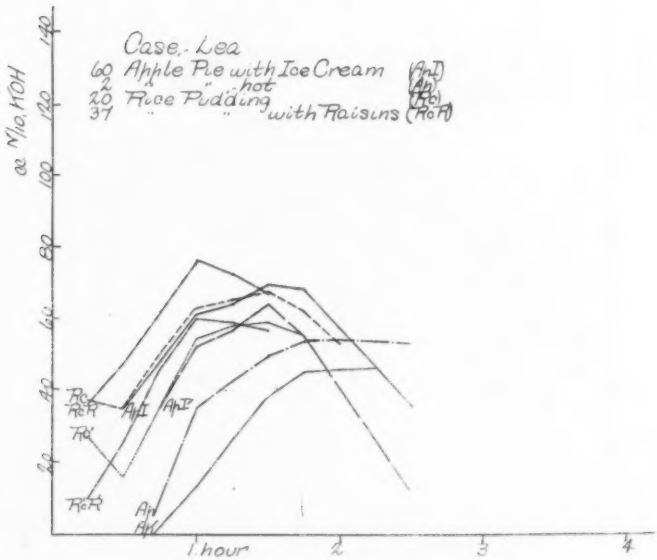
Case. Lea
60 Apple Pie with Ice Cream (ApI)
2 " " hot (ApI')
20 Rice Pudding (RcP)
37 " " with Raisins (RcP')
cc N/10 KOH
140
120
100
80
60
40
20
RcP
RcP'
ApI
ApII
Ap
Ap'
1 hour
2
3
4

FIG. 4

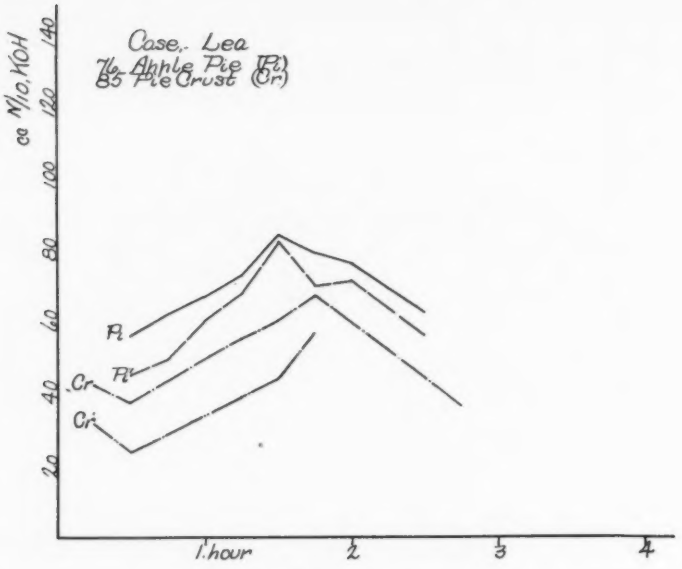
Case,- Lea
76 Apple Pie (Ap)
85 Pie Crust (Cr)
cc N/10 KOH
140
120
100
80
60
40
20
Ap
Ap'
Cr
Cr'
1 hour
2
3
4

FIG. 5

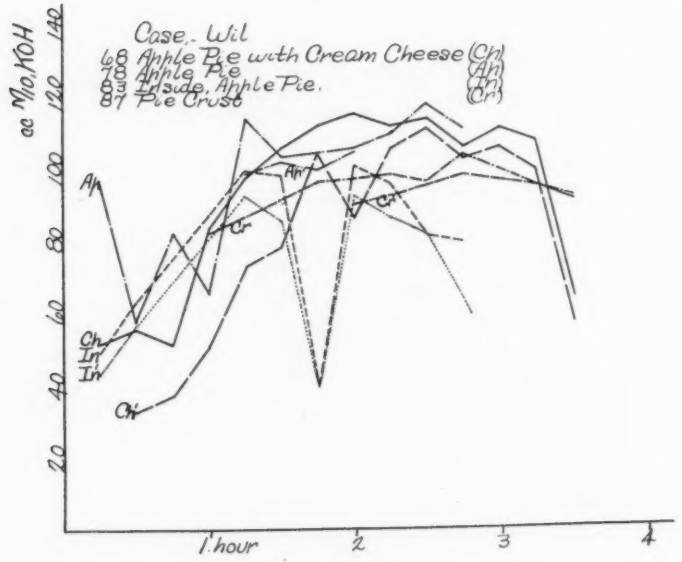
Case,- Wil
68 Apple Pie with Cream Cheese (Ch)
78 Apple Pie (Ap)
83 Inside, Apple Pie. (In)
87 Pie Crust (Cr)
cc N/10 KOH
140
120
100
80
60
40
20
Ap
Ap'
Cr
Cr'
Ch
In'
In
Ch'
1 hour
2
3
4

FIG. 6

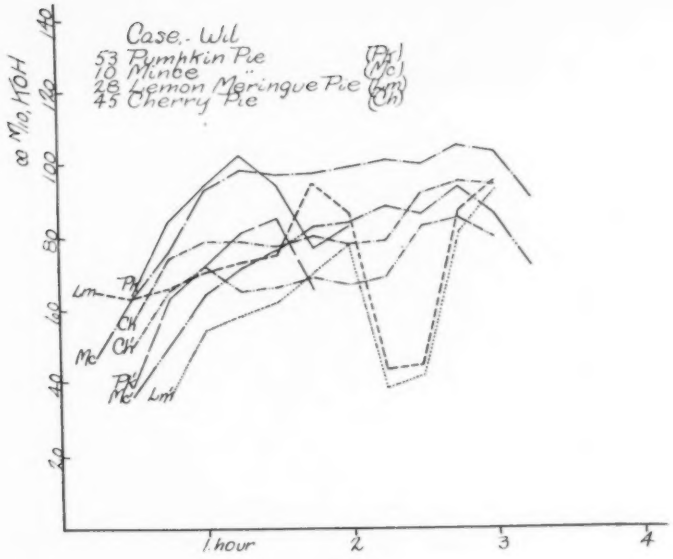
Case.- Wil
53 Pumpkin Pie (Pk)
10 Mince " (Mc)
28 Lemon Meringue Pie (Lm)
45 Cherry Pie (Ch)
cc N/10 KOH
1 hour
2
3
4

FIG. 7

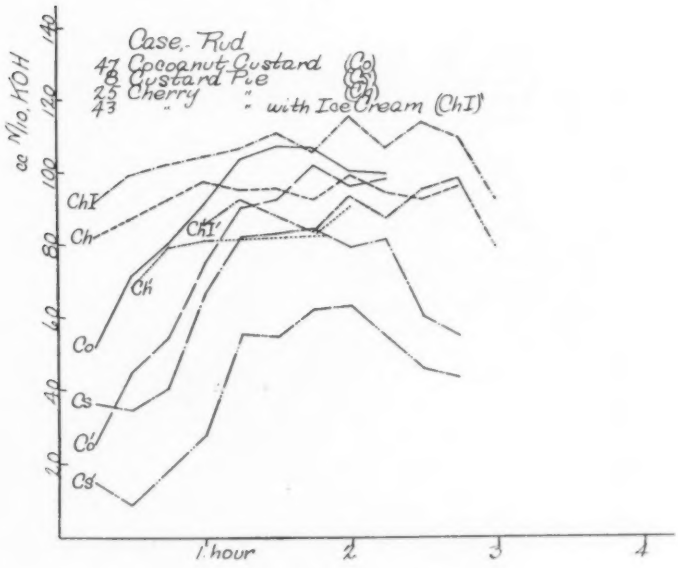
Case.- Rud
47 Cocoanut Custard (Co)
8 Custard Pie (Cs)
25 Cherry " (Ch)
43 " " with Ice Cream (ChI)
cc N/10 KOH
1 hour
2
3
4

FIG. 8

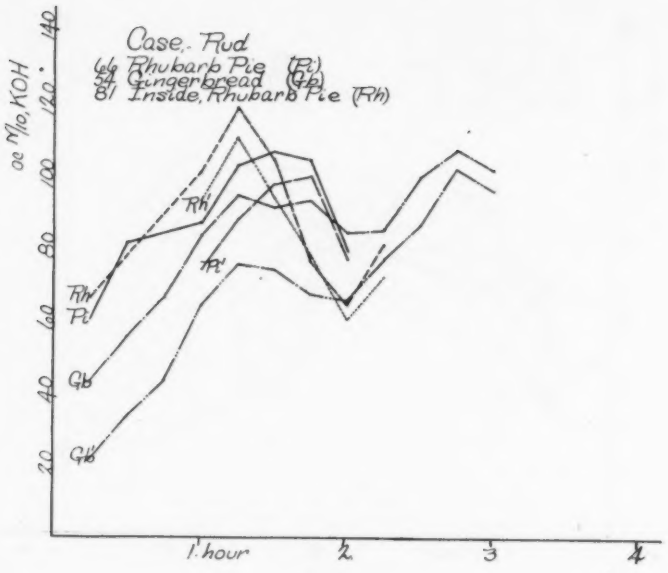
Case. Rud
64 Rhubarb Pie (Pi)
34 Gingerbread (Gb)
81 Inside, Rhubarb Pie (Rh)
cc N/10. KOH
140
120
100
80
60
40
20
Rh'
Pi'
Gb'
Rh
Pi
Gb
1 hour
2
3
4

FIG. 9

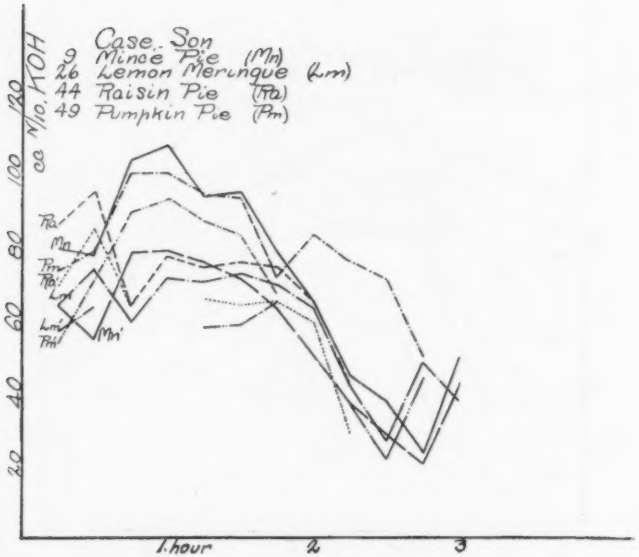
Case. Son
9 Mince Pie (Mn)
26 Lemon Merunque (Lm)
44 Raisin Pie (Ra)
49 Pumpkin Pie (Pm)
cc N/10. KOH
120
100
80
60
40
20
Ra'
Mn
Pm'
Ra
Lm
Lm'
Pm
Mn'
1 hour
2
3

FIG. 10

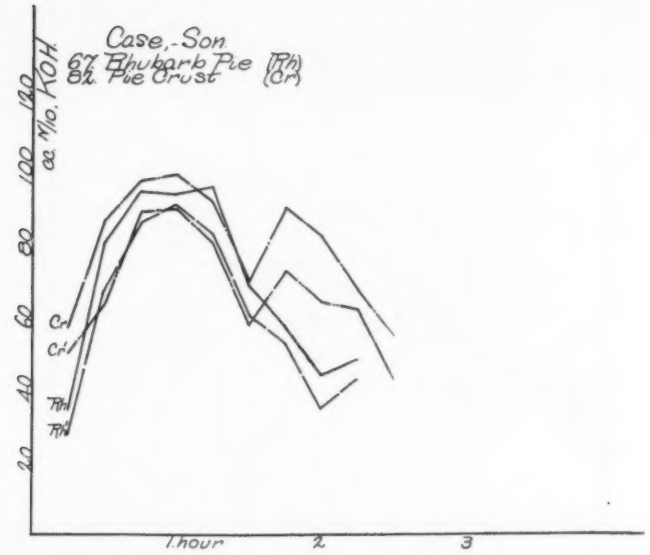
Case,- Son
67. Rhubarb Pie (Rh)
82. Pie Crust (Cr)
cc N/10 KOH
140
120
100
80
60
40
20
Cr
Cr'
Rh
Rh'
1 hour
2
3

FIG. 11

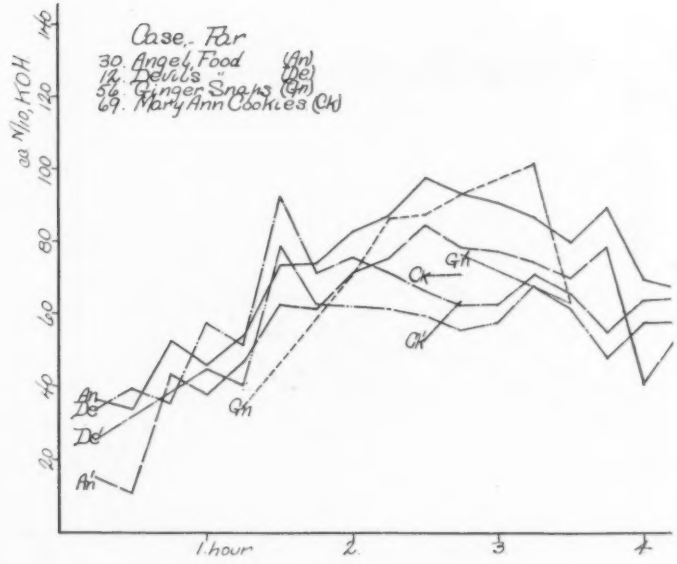
Case,- Far
30. Angel Food (An)
12. Devil's " (De)
56. Ginger Snaps (Gn)
69. Mary Ann Cookies (Ck)
cc N/10 KOH
140
120
100
80
60
40
20
An
De
De'
An'
Gn
Ck
Gn'
Ck'
1 hour
2
3
4

FIG. 12

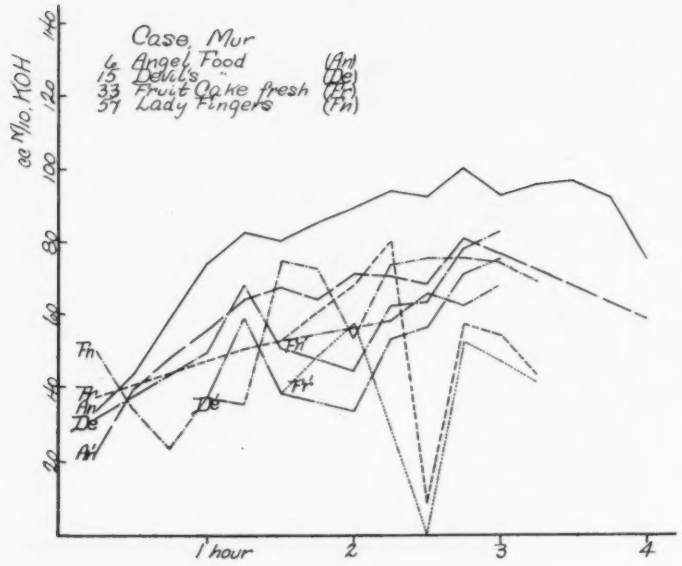
Case, Mur
4 Angel Food (An)
15 Devil's " (De)
33 Fruit Cake fresh (Fr)
37 Lady Fingers (Fn)
cc N/10 KOH
1 hour
2
3
4

FIG. 13

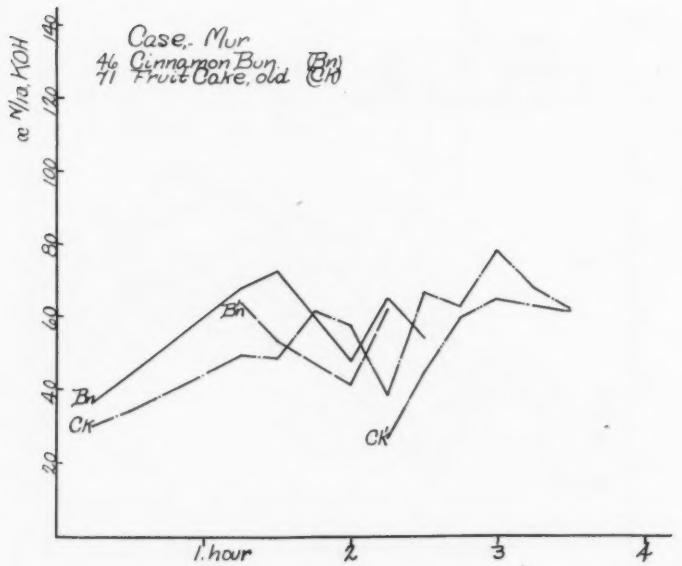
Case, Mur
46 Cinnamon Bun (Bn)
71 Fruit Cake, old (Ck)
cc N/10 KOH
1 hour
2
3
4

FIG. 14

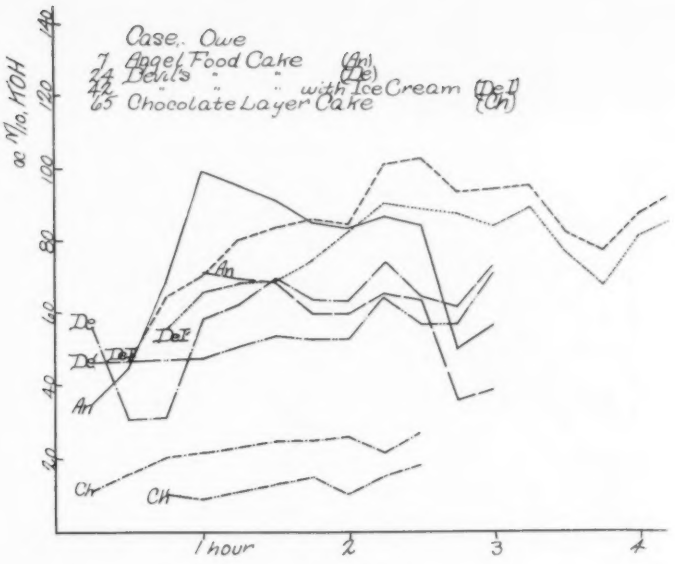
Case. Owe
7 Angel Food Cake (An)
24 Devil's " " (De)
22 " " " with Ice Cream (DeI)
65 Chocolate Layer Cake (Ch)
cc N/10 KOH
140
120
100
80
60
40
20
An
De
DeI
Ch
1 hour
2
3
4

FIG. 15

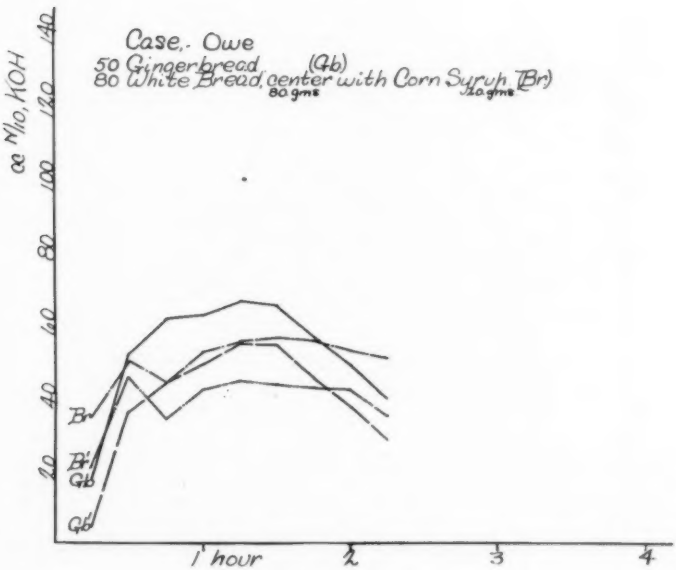
Case. Owe
50 Gingerbread (Gb)
80 White Bread, center 80 gms with Corn Syrup 10 gms (Br)
cc N/10 KOH
140
120
100
80
60
40
20
Br
Gb
1 hour
2
3
4

FIG. 16

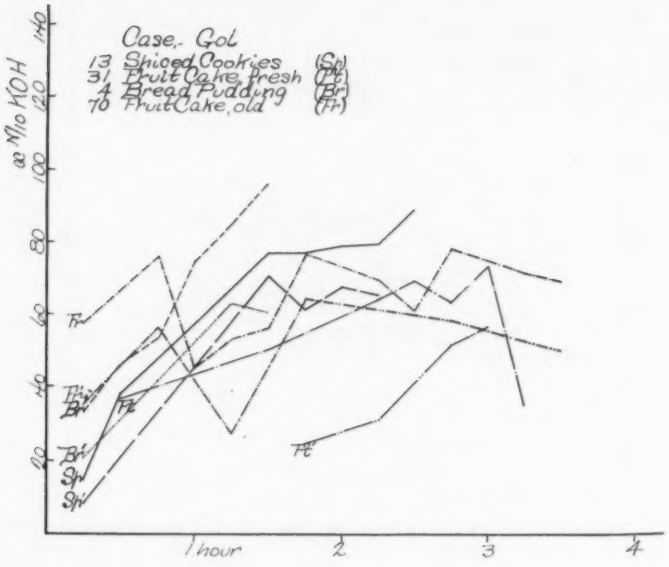
Case. Col
13 Spiced Cookies (Sp)
31 Fruit Cake, fresh (Ft)
4 Bread Pudding (Br)
76 Fruit Cake, old (Ft)
cc N/10 KOH
1 hour
2
3
4

FIG. 17

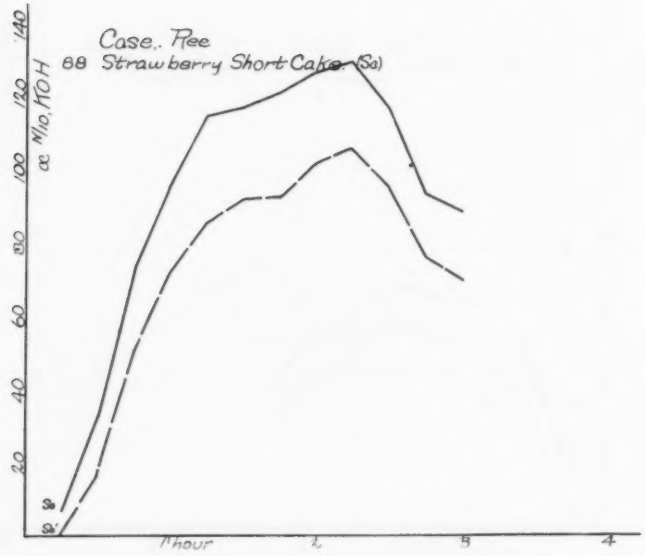
Case. Ree
68 Strawberry Short Cake (Sa)
cc N/10 KOH
1 hour
2
3
4

FIG. 18

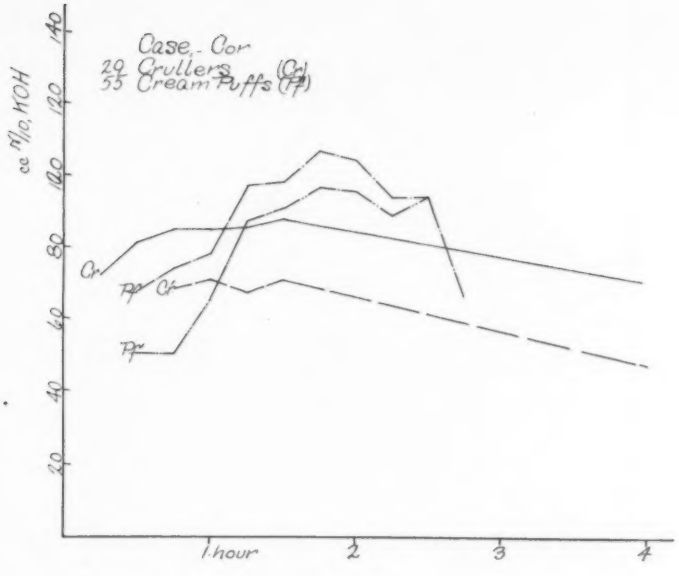
Case, Cor
20 Crullers (Cr)
55 Cream Puffs (Pf)
cc N/10 KOH
140
120
100
80
60
40
20
Cr
Pf Cr
Pf
1 hour
2
3
4

FIG. 19

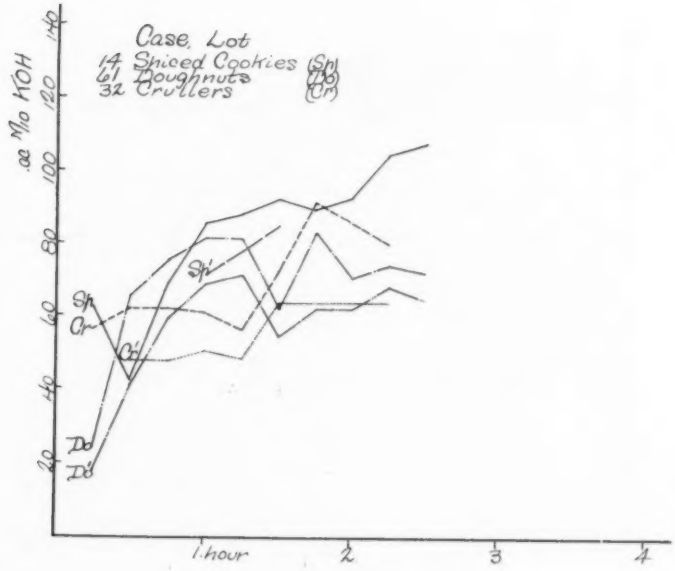
Case, Lot
14 Spiced Cookies (Sp)
61 Doughnuts (Do)
32 Crullers (Cr)
cc N/10 KOH
140
120
100
80
60
40
20
Sp'
Sp
Cr
Cr'
Do
Do'
1 hour
2
3
4

FIG. 20

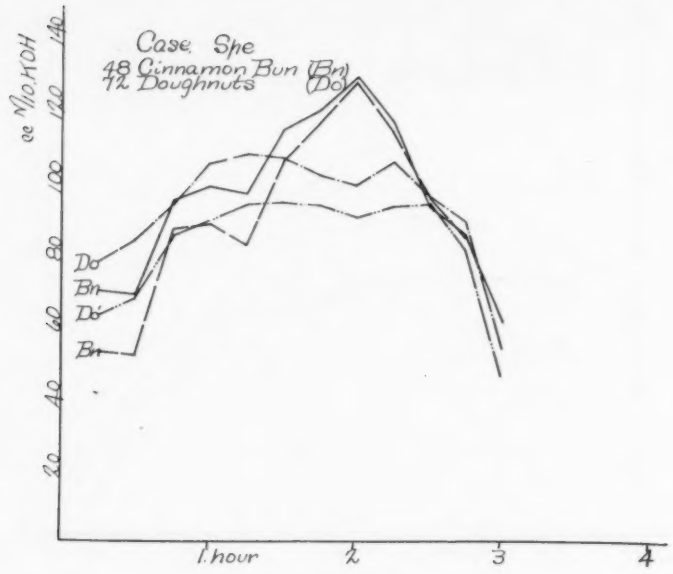
Case. Spe
48 Cinnamon Bun (Bn)
72 Doughnuts (Do)
cc N/10 KOH
140
120
100
80
60
40
20
Do
Bn
Do
Bn
1 hour
2
3
4

FIG. 21

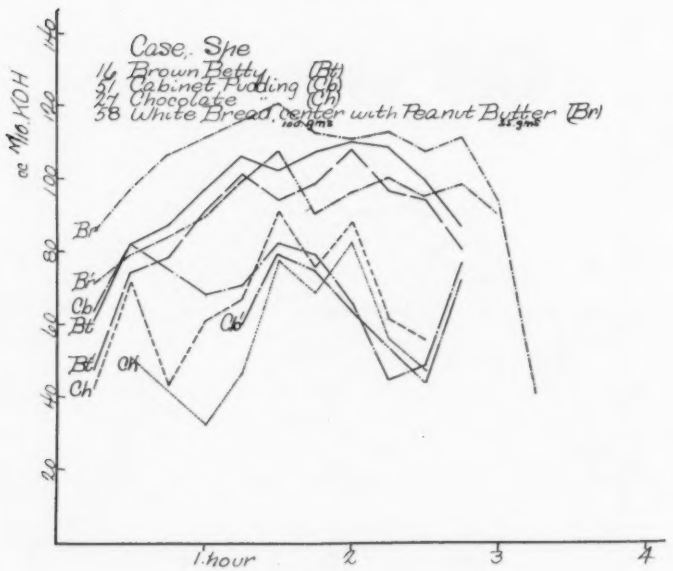
Case. Spe
14 Brown Betty (Bt)
37 Cabinet Pudding (Cb)
37 Chocolate " (Ch)
38 White Bread, center with Peanut Butter (Bn)
cc N/10 KOH
140
120
100
80
60
40
20
Bn
Bt
Cb
Bt
Ch
1 hour
2
3
4

FIG. 22

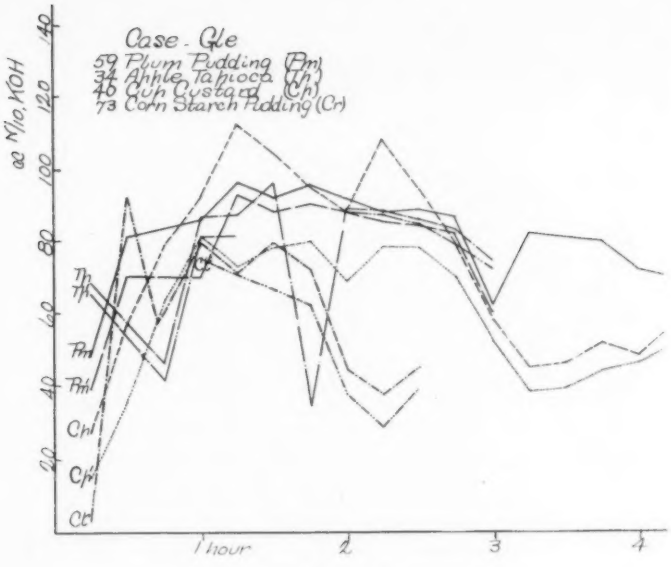
Case. Gle
59 Plum Pudding (Pm)
34 Apple Tapioca (Th)
46 Cup Custard (Cr)
73 Corn Starch Pudding (Cr)
cc N/10 KOH
1 hour
2
3
4

FIG. 23

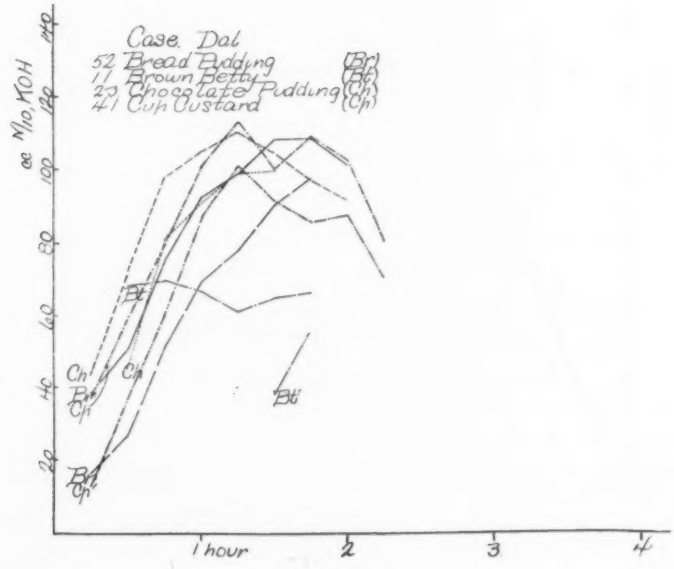
Case. Dal
52 Bread Pudding (Br)
11 Brown Betty (Bt)
20 Chocolate Pudding (Ch)
41 Cup Custard (Cp)
cc N/10 KOH
1 hour
2
3
4

FIG. 24

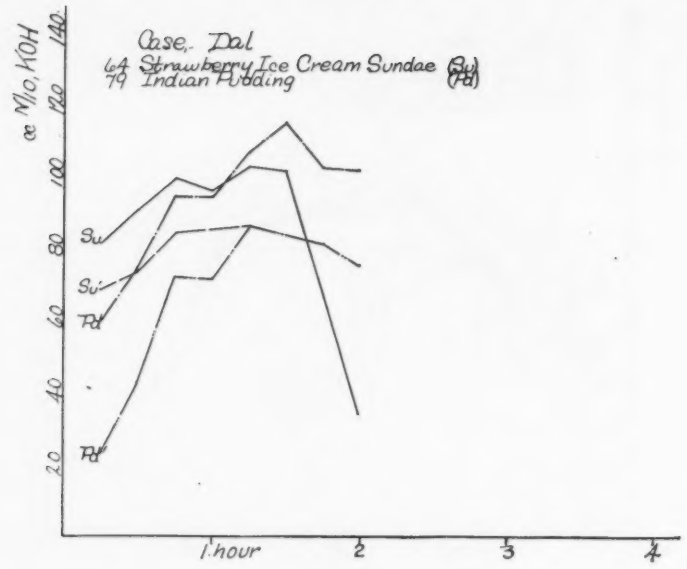
Case,- Dal
64 Strawberry Ice Cream Sundae (Su)
79 Indian Pudding (Pd)
cc M/10 KOH
140
120
100
80
60
40
20
Su
Su
Pd
Pd
1 hour
2
3
4

FIG. 25

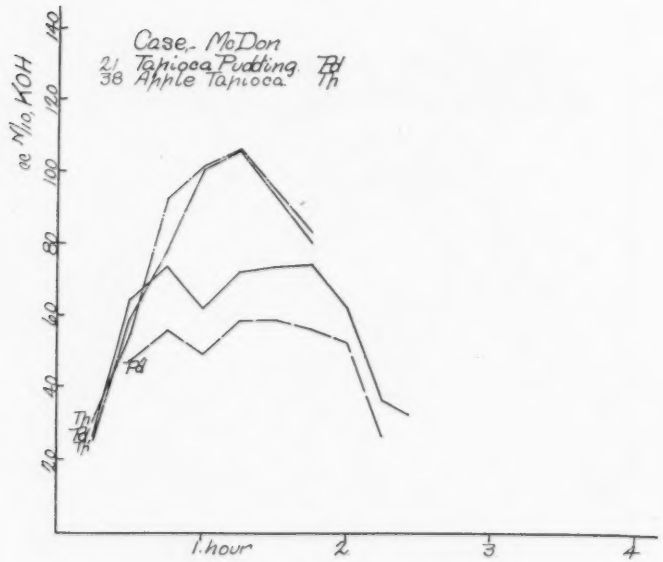
Case,- McDon
21 Tapioca Pudding Pd
38 Apple Tapioca Th
cc M/10 KOH
140
120
100
80
60
40
20
Pd
Th
Pd
Th
1 hour
2
3
4

FIG. 26

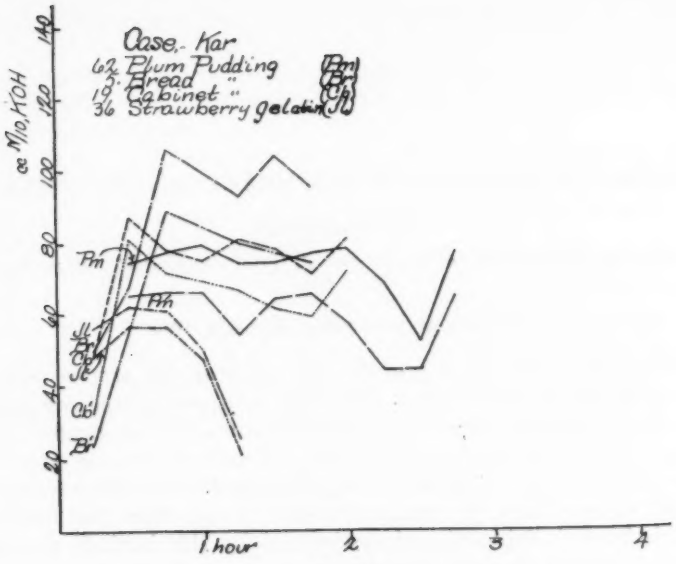
Case,- Kar
62 Plum Pudding (Pm)
19 Bread " (Br)
19 Cabinet " (Ca)
36 Strawberry Gelatin (St)
cc N/10 KOH
140
120
100
80
60
40
20
Pm
Ph
Br
Ca
St
Cb
Bi
1 hour
2
3
4

FIG. 27

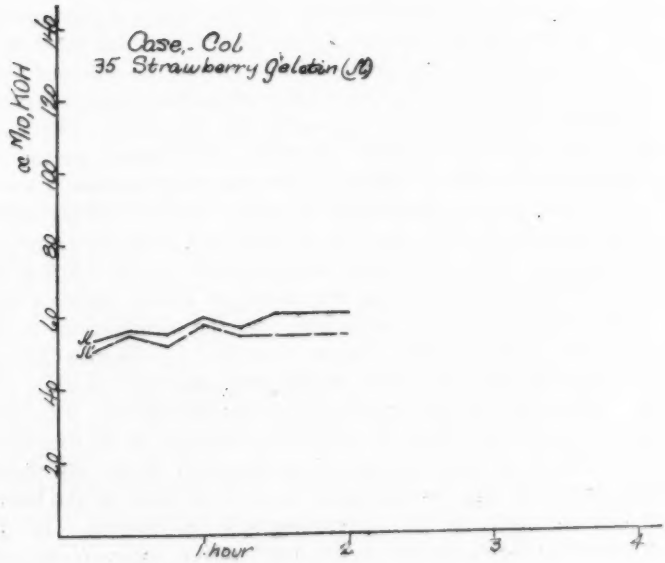
Case,- Col
35 Strawberry Gelatin (St)
cc N/10 KOH
140
120
100
80
60
40
20
St
Si
1 hour
2
3
4

FIG. 28

SEGMENTAL ACTIVITY IN THE HEART OF THE LIMULUS

D. J. EDWARDS

From the Physiological Laboratory, Cornell University Medical College, New York City

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In the structural make-up of the Limulus heart the arrangement of the muscular elements into a long tube with well-defined segments, and the grouping of the nervous elements into a median ganglion and lateral nerves, are features which have a significant bearing upon its functional activity. Attention is especially directed to these features, since it appears from the work of Carlson (1) and others that activity in this heart is initiated and conditioned by this intrinsic nervous system, and that contraction in all segments is practically simultaneous. These considerations have suggested the present study, which is an analysis of the contractile process in the different parts of the muscular tube, and of the sequence of activity in the different segments in an attempt to discover the exact temporal relations of the different parts.

In order to make a detailed study of the contraction event it is necessary to employ instruments of unquestionable efficiency. The shortcomings of the suspension method of recording heart action are too well known to need repeating; it suffices to note that records made from the heart of this form by arranging in various ways, so that a thread hooked into one of the lateral arteries may be attached to a cumbersome recording lever, gives a very inadequate representation of the contraction. Small myocardiographs recording by means of Frank capsules were used in the experiments here described, and the arrangement of the apparatus was essentially the same as described by Wiggers (2) and used for analyzing the contraction process in mammalian auricle. The method of adapting it to the Limulus heart was as follows: The myocardiograph arms were adjusted to a given segment so as to exactly span the width of the heart at the end of diastole. A fine silk thread was then passed through the superficial layer of the heart at the lateral borders and the arms of the myocardiograph tied thereto. In this manner there was slight traction on the heart until it began to contract.

Moreover, there was very little resistance offered to the shortening as the myocardiograph and also the Frank capsule were covered with very thin rubber membrane.

The record obtained is that of the mid-segmental shortening, the part of the heart that normally exhibits greater contractility than the inter-segmental portions where the ostea are located and in the anterior part the lateral arteries are attached.

The myogram of different segments. In considering the relation of the heart of the Limulus to its circulatory system it will be noted that the five anterior segments are each connected with outgoing pathways for the blood. If one reasons from analogy to the mammalian heart, a greater contractility should mark the myogram of these parts as compared with that of the posterior segments.

Fig. 1. Simultaneous myograms from heart segments 2, S_2, and 5, S_5, showing differences in the contour of the curve from these parts.

A comparison of the myogram from the two ends of the heart, for example, segments 2 and 8, shows differences in the contour, but the curve from segment 5, which has lateral openings, does not exhibit significant difference from that of segment 6, which has no such openings. It appears, therefore, that specialization in contractility, such as is shown in the ventricle of the mammalian heart, is not sharply delimited in the heart of the Limulus to that part from which the circulating fluid is outgoing.

The contraction amplitude is nearly always greatest in segment 2. The myogram of this segment, in figure 1, shows a small primary rise, *b* to *c*, upon which is superposed the main contraction, *c* to *d*. The upstroke of the principal rise is rapid and the summit is relatively

pointed, indicating that the state of maximal contraction occurs in all of the elements of this segment at practically the same instant. All of these features are exhibited in the myogram of segment 3, excepting that the degree of contraction is usually a little less. The myograms of 4 to 8 all show a smooth rounded contour and, barring differences in the amplitude, the curves from these different segments are practically superimposable. The shortening process in these segments develops slowly, as is shown fairly typically in the myogram of segment 5, figure 1, and moreover the maximal shortening is reached gradually, giving the curve a flattened summit. These features indicate that the contraction takes place in a wave-like manner; some of the elements at the beginning of systole are contracting while others are quiescent, while at the end of systole some are undergoing relaxation while others are still contracting. This form of contraction is suggestive of the type found in the mammalian auricle (2).

In the vigorously acting heart the myograms of segments 2 and 3 are characterized by a slowly developing state of tension preceding the beginning of contraction. This is shown in the myogram of segment 2, figure 1, in the part of the curve from *a* to *b*. The diastolic phase continues so that the curve passes somewhat below the level at the beginning of systole. Then follows the state of developing tonus during which the curve gradually swings back. In a slowly beating heart the curve reaches the level before the next systole begins, but with a more rapid rhythm the tonus increase extends right into the next systole. These presystolic tonus changes have not been found in hearts exposed for an hour or more to experimental procedures; they have also not been exhibited in the records from the posterior segments.

The sequence of segmental activity. Inasmuch as the experimental evidence for the Limulus heart favors the view that the impulse is initiated in the median ganglion, the precise time of activity in different segments is a matter of some interest. A casual inspection of a pulsating heart gives the impression of a simultaneous event throughout the nine segments. Carlson (3) states that "when an empty heart has been beating for several hours, or till nearly exhausted and the rate of the pulsations is in consequence much reduced, it can be made out . . . . that the contractions start in the posterior third of the heart and travel anteriorly. . . . . This is evidently the case in the fresh and vigorous heart." In a later communication the same writer (4), discussing the degree of automatism of different parts of this heart, attributes this function primarily to the middle third, in a less degree to the posterior third, and least in the segments of the anterior third.

From the foregoing statements it is evident that the question of segmental sequence can be answered only by exact measurements of initial activity in different segments. This has been done by simultaneous myograms of selected parts of segments, and by myograms related to the wave of excitation as detected by a string galvanometer. The usual procedure of taking simultaneous myograms was to attach one myocardiograph to segment 2 and a second to segment 3, then after a short interval records were taken. One myocardiograph was then changed to the next segment and the process repeated, and in a similar manner records were taken from all segments. Usually only one series was taken from a heart and sometimes only a few segments were compared, but each record was taken in duplicate and the mean of these was used in making up the data for that experiment.

In general the results show that the middle segments, that is, segments 4 and 5, exhibit activity in a given cycle earlier than the posterior or anterior segments. A comparison of the beginning of contraction of segment 5 and of segment 2 in sixteen experiments shows that segment 5 preceded by an average interval of 0.046 second. In nine experiments comparing segment 2 with segment 4 there is shown an average time interval of 0.047 second, with segment 4 preceding. This indicates that the beginning of contraction in segments 4 and 5 is practically simultaneous and that it normally precedes the contraction of segment 2. On the other hand, a comparison of segment 2 with segment 6, for example, shows in eight experiments an average difference of 0.023 second, indicating that while segment 6 slightly precedes the beginning of contraction of segment 2, it follows by an average interval of 0.024 second the contraction of segment 4.

A comparison of the initial activity in segments 7 and 8 by this method has given less satisfactory results because of the usually low degree of contractility which makes it difficult to determine precisely the beginning of shortening. It is possible, therefore, to make only a general statement concerning them. In six experiments comparing the beginning contraction of segments 2 and 7, it is shown that segment 7 precedes that of segment 2 in four instances and follows it in the remaining two. In a similar way, the data for seven experiments comparing segments 2 and 8 show that the contraction of segment 8 preceded the contraction of segment 2 in three instances and followed it in the remaining four. This indicates that the contraction of the posterior segments normally takes place at practically the same time as the anterior ones.

In order to test the matter of segmental sequence in another way, the excitation process of different segments was taken with a string galvanometer and in turn related to the myogram of a particular seg-

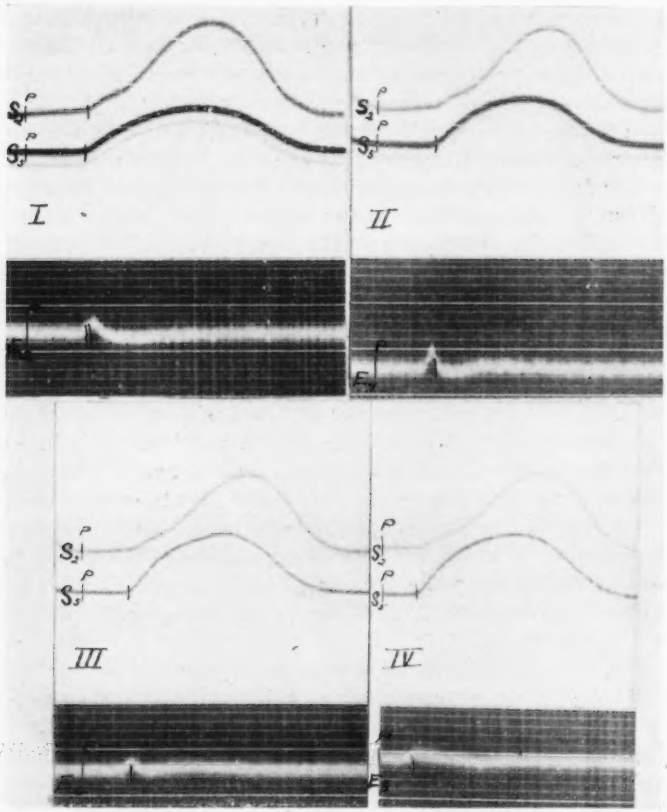

Fig. 2. Electrocardiogram recorded from segments 2, *I;* 4, *II;* 6, *III*, and 8, *IV*, to show time relation of excitation in these parts to the contraction of segments 2 and 5. Position of points indicated by *P*. Ordinates in bottom portion of curves designate time intervals of 0.20 second.

ment. The current was led off by placing one electrode on part of the dorsal muscle and the other beneath the heart so that the segment under observation rested upon it.

The characteristic form of the electrocardiogram for this heart has been very well described by Hoffmann (5); little attention, therefore, was given to this feature. In the experiments reported here the beginning of deflection was taken as the index of initial activity, and in figure 2 is shown a series of records which illustrate the time relations for different segments in a fairly typical way by this method. Curve *I* shows that excitation in segment 2 precedes the beginning of shortening in this segment by 0.08 second, and by 0.05 second the initial recorded shortening in segment 5. In curve *II* of this figure it is shown that excitation of segment 4 precedes the contraction of segment 5 by 0.09 second. In a similar manner it is determined from curve *III* that excitation in segment 6 precedes the contraction of segment 5 by 0.06 second, and from curve *IV*, that excitation in segment 8 precedes contraction of segment 5 by only about 0.03 second. Relating these data, it appears, therefore, that the excitation of the median segments takes place about 0.04 second before excitation of the anterior segments and about 0.03 second before excitation of the posterior segments. In this connection it is interesting to note that the distance separating the electrode contact on segment 4 and that on segment 2 was 29 mm., and the time difference in the sequence of activity of these segments, as related to the myogram of segment 5, was 0.04 second. It is evident from these data that the rate of conduction was approximately 72 cm. per second, a figure that is significant in view of the average rate of 40 cm. per second for the conduction rate in the Limulus heart nerves, as obtained by Carlson (6) with less sensitive methods.

Mention should be made of the condition met with in some experiments in which the anterior segments showed unmistakably an earlier state of activity than the middle segments. We have in figure 3, for example, a record of contractions of segments 3 and 5 showing the contraction of section 3 preceding by 0.05 second. This proved to be a constant feature of the contraction of this particular heart, since other records in this experiment comparing segments 1 and 6, and 1 and 5, show the same general result. Of the thirty-three experiments in which records have been made this phenomenon has been observed to occur with a degree of constancy in only four hearts. It is significant, however, that records taken from the same heart late in the experiment, after a considerable period of exposure, show initial activity in the median segments.

A careful study of initial activity in the different segments of the Limulus heart makes quite evident that the distribution of the excita-

tion process is a rapid event, and that it normally encounters no marked areas of resistance which slow the excitation wave in its spread from the middle region of the heart. Garrey (7) has very clearly demonstrated, however, that this heart is susceptible to the usual types of blocking influences. Moreover, it is a fact well known to all who have worked with this heart that an adequate stimulus applied to any part starts a contraction wave which passes in either direction.

The evidence presented here supports the conclusion, I believe, that normally the contractions of the middle segments precede those of both anterior and posterior segments. The initial tonus changes often

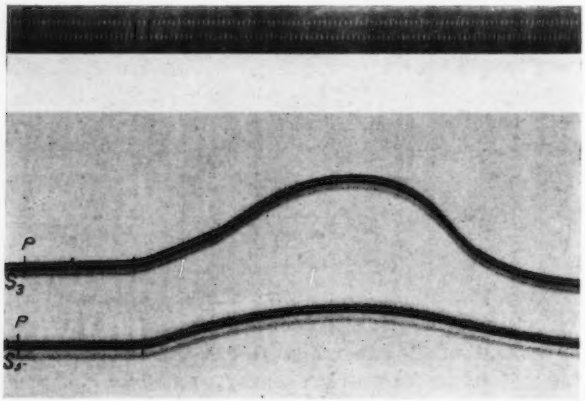

Fig. 3. Myograms from heart segments 3, S_3, and 5, S_5, showing an instance in which contraction in the anterior segments preceded that of the middle segments. Position of points indicated by P. Time curve designates 0.02 second for each full vibration.

exhibited in segments 2 and 3 are significant. It is believed that the increased tonus effects a more quickly acting mechanism in this part of the heart and, coupled with the high state of irritability in a vigorous heart, the recorded activity of this part may precede the initial activity of the relatively less contractile middle segments. The observation of Carlson that a heart, after beating for some time under experimental conditions shows a marked lagging behind of the anterior segments, has been corroborated. But this is taken to mean that the exposure, with the resulting decrease in the nutritive supply from loss of the circulating fluid, favors an earlier appearance of fatigue and blocking in-

fluences in the anterior part because these segments exhibit normally a greater degree of contractility and, therefore, a relatively more rapid using up of the stores of energy.

It has been pointed out in the study of the myogram from different segments that the function of contractility is exhibited in a diminishing degree in the anteroposterior direction; that the heart as it approaches exhaustion shows initial activity in the posterior segments, is interpreted not as the normal sequence of activity but as an expression of the change in irritability which accompanies muscular exhaustion.

SUMMARY

1. Myograms, made with small myocardiographs, from different segments of the Limulus heart, show greatest contractility in segments 2 and 3 and a diminishing degree posteriorly. The median and posterior segments give evidence of a more gradual development of the shortening of the muscular elements than is shown for the anterior segments, and similarly the end of the contraction process is less marked.

2. The sequence of activity in different segments, by the method of simultaneous myograms and by the method of relating the excitation wave to a standard myogram, indicates initial activity in segments 4 and 5 from 0.04 to 0.05 second before that of segment 2, and in segment 6, about 0.03 second before that of segment 8.

3. In a fresh and vigorous heart there is exhibited a presystolic tonus increase in segments 2 and 3 which appears to hasten the contractile event in these parts so that at times it occurs simultaneously with or even precedes the contraction of the middle portions.

I wish to acknowledge my indebtedness to the Director of the Marine Biological Laboratories for working facilities and to the Director of the New York Aquarium for supplying a part of the material.

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(4) CARLSON: This Journal, 1904, xii, 471.
(5) HOFFMANN: Arch. f. Physiol., 1911, 142.
(6) CARLSON: This Journal, 1905, xv, 101.
(7) GARREY: This Journal, 1912, xxx, 283.

THE ARTERIAL PRESSURE CURVE AS INFLUENCED BY THE OCCLUSION OF CERTAIN VASCULAR AREAS AND BY HISTAMINE

D. J. EDWARDS

From the Physiological Laboratory, Cornell University Medical College, New York City

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It has been shown in a number of researches by Wiggers (1) that the contour of the pressure curve recorded by the membrane manometer affords reliable qualitative data on the dynamics of the circulation. An outstanding feature of this method is that it gives information concerning the failure of normal dynamic relations earlier than the blood pressure results. It has made possible, therefore, a fairly precise designation of the onset of circulatory failure (2).

In the course of studies on blood flow (3) the use of a recording stromuhr made it necessary to close for periods of as much as twenty minutes the main blood vessels supplying visceral organs and in other instances those supplying the extremities. Roy and Brown (4) were probably the first to call attention to the fact that occlusion causes in the frog a dilatation of the arterioles to twice their normal size in two minutes, the effect extending also into the capillaries and veins. Recently attention has been directed to the possible harmful tendency of occlusion measures by the experiments of Mann (5) in which he shows that a shock-like condition can be produced by ligating all of the limb structures excepting the main artery, and Erlanger and Gasser (6) have shown that a similar condition results from a partial obstruction of the inferior cava for about a 2-hour period. In view of these results it is obviously desirable to know whether the less extreme measures used in blood flow experiments produce early changes in the dynamics, also whether there are differences in the character as well as the time of appearance, which distinguish obstructions of central areas from those involving peripheral organs.

There has been included in the program a study of the influence of histamine on the pressure curve, since the observed capillary toxicity of this product suggested a causal relation to the effects following occlusion of vascular areas.

METHOD

The pressure curve in all experiments was taken by introducing the cannula of the optical manometer into the carotid artery as low down in the neck as possible. A record of mean blood pressure was taken by connecting a mercurial manometer to the opposite carotid artery. This necessitated the closure of two principal arteries but it has been shown that the free anastomosing of the vertebral and spinal arteries permits of compensation through these channels.

Dogs weighing 8 to 10 kilos were used, anesthetized with ether and maintained by closed method with especial care to keep the degree of narcosis very light throughout.

Four different methods of occlusion were made use of: *a*, Ligating of three extremities by placing the ligature about the appendage in such a manner as to exclude the main artery; *b*, clamping of the principal veins to three extremities; *c*, clamping of the inferior mesenteric vein; and *d*, clamping of the inferior cava above the level of the renal veins. In applying the clamp to the inferior mesenteric and the inferior cava a small right abdominal incision was made through which these vessels were exposed and especial care was taken to disturb the viscera as little as possible.

Ligation of the extremities and clamping of the principal veins to these parts: In the experiments where ligation effects were studied the extremities were enclosed in the ligatures for a 20-minute period, then for 5 minutes the ligatures were released and in this interval records were usually taken at the beginning and end. The ligatures were then applied again and the procedure repeated. In other experiments clamps were placed on the principal veins and these were manipulated according to the procedure given for the ligatures. The methods differ, therefore, partly in that clamping of the veins does not block certain anastomosing pathways close to the trunk, but mainly in that clamping does not produce tissue injury as does alternate application and release of the ligatures.

The immediate changes when the circulation is reëstablished in the extremities are not essentially different in ligating from that when clamps were applied to the principal veins. Usually there is a fall in pressure of not more than 10 mm. mercury with gradual recovery during the 5-minute interval; sometimes there is shown a sharp initial rise and then a fall. The optical tracings taken at the beginning and end of this period show no significant changes.

The records, when viewed as a whole for the seven experiments of the series and the successive stages in each compared, show moderate changes after about 3 hours of the alternate occlusion and deocclusion. All of the experiments were continued for approximately 4 hours. The final contour of the pressure curve usually takes the form of a marked primary oscillation followed immediately by a sharp decline of the curve replacing the systolic plateau of the normal curve. These features are quite characteristically shown in the three segments reproduced in figure 1, in which corresponding points in the curves are indicated by similar letters. These curves were taken as follows: *I* at the outset of the experiment; *II* at the end of 2 hours and 20 minutes during which the alternate 20-minute occlusion and 5-minute release of two femorals

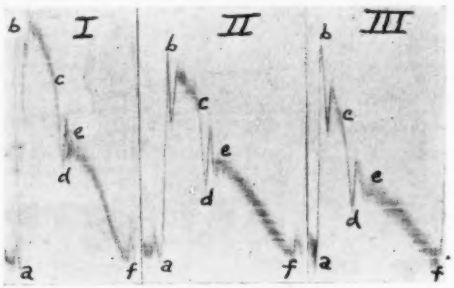

Fig. 1. Segments of the optical arterial curves during the progress of successive occlusion and deocclusion of the veins of three extremities.

and one brachial veins were carried out; *III* at the end of 3 hours and 40 minutes of alternate occlusion and release.

The change in mean blood pressure taking place in this period is slight, but significant in view of the fact that the directional change is downwards and that the optical curve is indicative of lowered peripheral resistance. The following table will assist in bringing out this feature of the blood pressure change and also the time of appearance of a marked change in the form of the pressure curve. This table shows that averaging the interval before a marked change was apparent in the contour of the pressure curve there was required about $3\frac{1}{2}$ hours of alternate clamping of the veins or ligating of the extremities and that much longer time was necessary to give unmistakable signs of decreased peripheral resistance. Definite indications of circulatory failure and

a shock-like condition were shown, however, in only one instance, that of experiment 8, but the data for this experiment give ground for the view that the occlusion effects per se played a relatively less part than trauma of the sensory nerves from application of the ligatures. There were marked sensory signs with each application of the ligatures and mean blood pressure in the early part of the experiment rose from 172 to 206 mm. Hg.; then occurred a fairly sudden break and a fall in pressure. The optical curve does not give indication of increased resistance as the main cause of this rise; it is probably due, therefore, to an increased cardiac output.

Thus, however viewed, the alternate occlusion and deocclusion of the circulation of three extremities fails to effect changes in the dynamics of a more pronounced character than have been shown to occur in the

TABLE 1

EXPERIMENT	BLOOD PRESSURE		LENGTH OF EXPERIMENTAL PERIOD		ONSET OF CHANGE IN PRESSURE CURVE		STATE OF ARTERIAL RESISTANCE AT FINISH
	At the start	At the finish					
			hours	*minutes*	*hours*	*minutes*	
1	106	110	5	50	3	45	Decreased
2	74*	118	3	45	3	15	Decreased
8	172	88	4	30	3	25	Very low
10	132	114	3		2	10	Decreased
11	152	132	3	50	3	20	Decreased
14	166	112	5		3	45	Decreased
15	126	112	4				No change

initial stages of circulatory failure of shock (2) and a period well over 3 hours is required for this change to appear.

Clamping of the inferior cava: The moderate degree of change in the pressure curve resulting from occulsion of the circulation of the extremities suggested extending the procedure to include larger vascular areas. It is interesting to notice in this connection that the minute volume flow to the posterior extremities, as calculated from the flow in the femoral vein (7), is 45 cc. There are no data available concerning the supply to the anterior extremity but it is usually assumed to be approximately equal to that of the posterior extremity. The three extremities, therefore, would represent a volume flow of 135 cc. per minute. On the other hand, it has been shown that the kidneys have a minute volume flow of 195 cc. for the two organs (8). These data represent average normal values and at best are only approximate,

but they serve to indicate that the clamping of the cava affects a many times greater vascular field than was included under the experiments on three extremities.

The pressure changes following cava obstruction indicate in a striking way that there is a greatly diminished venous return which causes some cardiac embarrassment. There is a reduction in the amplitude of the curves with a retardation in the gradient of the upstroke and the end of systole is not sharply marked by a distinct incisura. With the first deocclusion of the cava there is evident in the pressure curve a resumption of the main characteristics of the original, but after the second period of clamping little tendency to recovery is shown; furthermore, there is indicated a marked relaxation in peripheral tone. The total time of occlusion of the inferior cava necessary to give these indications of a break in the dynamics was never more than 1 hour. It may have been somewhat less since the periods of occlusion were from 25 to 30 minutes.

The results of one experiment in which the clamp was applied just posterior to the inlet of the renal veins is of interest, since in this manner the kidney circuit is left intact. The blood pressure fell about 30 mm. Hg. when the clamp was on but showed practically complete recovery each time it was removed. This was repeated four times, covering a period of 2 hours and 10 minutes, and at the end of this time the optical curve showed the characteristics of initial failure. There is suggested, therefore, a degree of proportionality between the time of appearance of these circulatory changes which influence the form of the pressure curve and the extent of the vascular field occluded

Occlusion of the inferior mesenteric vein: The inferior mesenteric vein drains an entirely visceral area in contrast to the conditions considered above. Moreover, it represents a vascular field as indicated by the figure for blood flow of 164 cc. per minute (9) quite comparable to that studied under occlusion of the extremities.

The outstanding features in the pressure curve during the progress of this type of occlusion are the relatively sudden appearance of the change in the contour of the curve and the slight indication of progressive development after this initial change. The first period of clamping was about 40 minutes and the pressure curve shows (fig. 2, *II*) at the end of this time a sharp primary oscillation (*a–b*) without plateau (*b–c*) and with low diastolic limb (*e–f*). Each record was taken at the end of the 5-minute period of deocclusion. Segments *III* to *VI*, while differing somewhat in amplitude, show slight changes in their essential

characteristics and give evidence of a comparatively fixed state of dilatation after the first period of stasis. Furthermore, these succeeding periods of occlusion show only moderate depression in blood pressure and nearly identical recoveries during the periods of deocclusion. These changes lend support to the view that the cardiac mechanism is not markedly affected by the degree of reduction in the venous return.

The significant features in these results appear, therefore, as the rapid initial decrease in blood pressure with comparatively small changes from the alternate periods of occlusion and deocclusion, and the slight tendency to a progressive type of change in the pressure curve. Many factors are concerned probably in the production of the effects following occlusion of the venous return from such vascular areas, but the

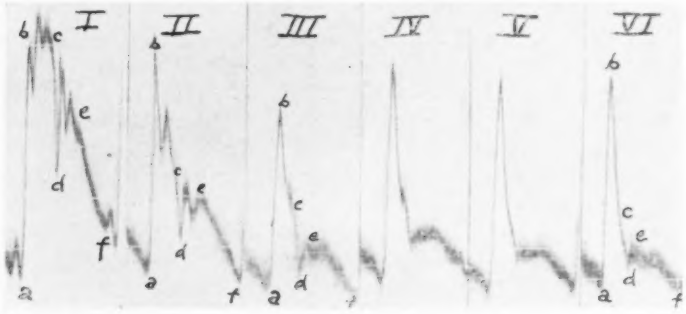

Fig. 2. Segments of optical arterial curves during the progress of successive occlusion and deocclusion of the inferior mesenteric vein.

following warrant special consideration: (*a*) Mechanical distention of the blocked venous system; (*b*) a toxic action of products formed in the stagnant areas which exert further injurious effects when periodically flushed into the circulation; and (*c*) the damaging of the vascular walls with accompanying alterations in permeability. The present experiments do not permit of direct conclusions concerning which of these factors plays the essential rôle in the production of the effects studied. Indirectly they give evidence, however, in favor of the view of mechanical dilatation as the main cause of these primary changes, since it has been shown that occlusion of an intestinal area which affords by virtue of its plastic character a favorable site for mechanical stretching of its vascular walls, is immediately affected and the change appears almost in full magnitude at the outset. The occlusion of a peripheral

field of comparable vascular magnitude, which presents a comparatively rigid vascular area, gives results essentially different in that corresponding changes in the pressure curve appear much later in the course of events.

Thus far, no account has been taken of the possible toxic effects of substances formed during the periods of stagnation. In order to throw light directly upon the probable degree of influence which substances of this character exert the experiments below with histamine were performed.

Successive injections of histamine: The results of Dale and Laidlaw (10) show quite conclusively that the administration of histamine may produce a condition simulating traumatic shock. This phenomenon

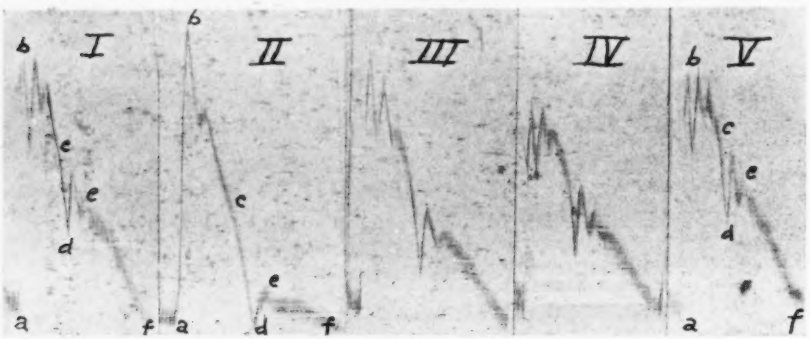

Fig. 3. Segments of optical arterial curves at certain stages during the blood pressure change from injection of 0.15 mgm. histamine. The numbering of segments corresponds to positions indicated on figure 4.

they assign to increased capaciousness through the relaxation of capillary tone from the toxic action of this substance. In their experiments cats were used for the most part and a dosage of a milligram per kilo body weight was found necessary to produce circulatory collapse.

In the present experiments on dogs it was sought to test not only the immediate effects but also the accumulative action of histamine on the dynamics of the circulation; accordingly, the injections were usually 0.04 or 0.05 mgm. (the ergamine of Burroughs, Wellcome & Co.) repeated at 10-minute intervals over a period of 3 to 4 hours. Such a dosage never fails to give at each injection a fall of over 50 per cent in blood pressure at the start. The recovery is rapid at first and usually complete in less than 3 minutes but as the experiment progresses and

also following larger injections a somewhat longer time is required before the pressure returns.

In figure 3 are shown portions of the optical records taken during the changes produced by the injection of 0.15 mgm. histamine. These curves, taken in connection with the tracing of the blood pressure change shown in figure 4, portray the immediate effects of this substance upon the pressure relations. Coincident with the fall in mean blood pressure the optical curve shows (fig. 3, segment *II*) a large primary oscillation

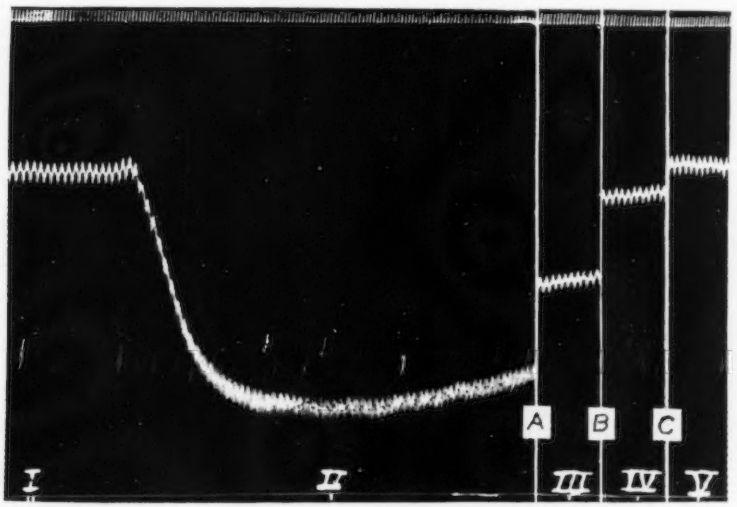

Fig. 4. Tracing showing blood pressure change as related to optical curves in figure 3. Numerals correspond to segments in that figure. The intervals *A*, *B* and *C* correspond to 2 minutes, 5 minutes and 5 minutes, respectively. Time curve indicates seconds.

(*a–b*), indicative of a collapsed state of the arterial system (*c–d*), and a flattened diastolic limb (*e–f*), signifying a low state of peripheral resistance. There is a rapid restoration of peripheral tone indicated in segment *III*, *e–f*, by the immediate rise in this portion of the curve. This change in resistance suffices to restore mean blood pressure in a short time but it will be seen from segment *IV* that the recovery of the dynamic relations is complete before mean blood pressure has reached its original level. The extent to which cardiac output is influenced by

these changes does not appear from the present data. Further experiments dealing with this feature are in progress.

Turning now to the influence of the successive injections, it was demonstrated at the outset that small doses, e.g., 0.01 mgm. per injection, have little accumulative effect. With individual injections in amounts of 0.05 mgm. it has been possible generally to produce in dogs of about 8 kilos signs of initial circulatory failure after a total of 0.5 to 0.7 mgm. had been injected in the manner indicated. The stages in the failing dynamics are very well brought out in figure 5. Segment *I* was taken at the outset of the experiment and serves as a basis for comparison; segment *II* was taken after seven 0.04 mgm. injections had been given; and segment *III*, after thirteen such injections. Close

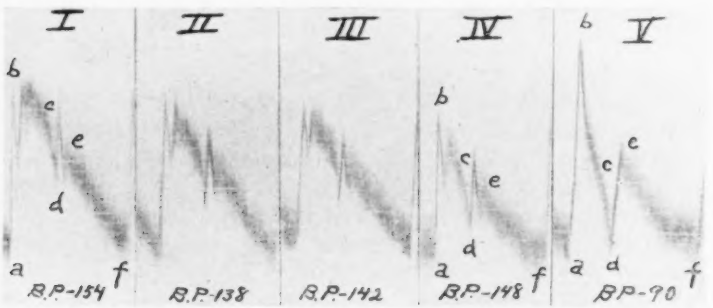

Fig. 5. Segments *I*, *II*, *III* and *IV*, optical arterial curve during progress of successive injections of histamine. Segment *V*, taken 25 minutes following injection of 5 cc. adrenalin (1:5000). Description in text.

inspection of these curves shows a sharper contour in *II* and *III* and a slight diminution in the height of the primary oscillation. These features are significant in that they anticipate the more marked changes characteristic of failing peripheral resistance and that they take place without significant changes in mean blood pressure. At this stage of the experiment the dosage was increased. There followed two 0.2 mgm. injections at the usual 10-minute intervals and one injection of 0.5 mgm. Whereas the smaller amounts had usually caused a fall in blood pressure to 50 to 60 mm. Hg., the latter dose depressed the pressure to 36 mm. Hg. Segment *IV* was taken 20 minutes following the 0.5 mgm. injection and at a time when blood pressure had practically recovered. It will be seen that the introduction of the larger amounts augmented the features already noted and that the curve now exhibits

a certain likeness to that shown under the occlusion of the veins to the extremities and characteristic of initial failure.

These results present quite typically the effects on the optical curve of repeated injections of histamine. It is significant to note, also, that all of the experiments were continued for a period of about 4 hours without a single instance of circulatory collapse appearing, notwithstanding injections at the conclusion of some of the experiments of a milligram of histamine following closely upon successive doses only slightly less, with a total given during the experiment amounting to 3 mgm. or more. These observations lend support to the view that the dilatation resulting from the amounts of histamine used, and administered in the manner indicated, causes only moderate dynamic changes. Furthermore, it appears that the change is one that may be due to the reduced resistance from simple capillary dilatation. With the possibility in mind that a moderate vasoconstriction might counteract the effects of the periodic histamine dilatations and thereby restore the failing dynamics, injections of 5 cc. of adrenalin (1:5000) were given at the rate of about 1 cc. per minute. The usual constrictor response followed but passed off in ten to fifteen minutes and a vascular equilibrium was again established. The pressure relations at this stage are very well shown in segment *V* of figure 5, taken 25 minutes following the administration of the adrenalin. It shows that, notwithstanding a good state of peripheral resistance signified by the height and slope of the diastolic limb, *e–f* of the curve, there is a diminished distention of the arterial system evidenced by the increased amplitude and sharp contour of the primary oscillation, *a–b*.

The marked vasoconstriction of the adrenalin appears to have the effect, therefore, of augmenting the condition started by the histamine. In this connection it is interesting to consider briefly the possible mechanism of action of adrenalin whereby it accentuates the dilatation initiated by histamine. Hartman and McPhedran (11) state that large doses of adrenalin dilate the intestinal vessels. On the other hand Erlanger and Gasser (12), while not able to agree with this conclusion, point out that portal pressure is greatly increased by giving large amounts.

The results presented above give unmistakable evidence of a decrease in peripheral resistance after repeated small doses of histamine. It is well to bear in mind that several factors may contribute to this end, important of which are decreased arterial tone, dilatation of the capillary areas and decreased viscosity of the blood. While it is not possible

to state with certainty regarding which of these factors plays the greater rôle in the reduction of peripheral resistance with histamine, it is reasonable to assume that the action of adrenalin described above is due to a further dilatation of the weakest link in the vascular circuit. The usual absence of reduction in arterial tone from adrenalin, and also the excellent results of Dale and Richards (13), indicating that histamine is a capillary dilatant; support the conclusion that the capillary system is the part less resistant and is the area which compensates for the intense constriction of the adrenalin. The data at hand do not indicate whether this effect is mainly on the intestinal area or is generally present in the capillary blood-bed of all organs. The observation of a great increase in portal pressure lends support to the view that the area supplied by this vessel may play the larger part in these results.

One point still remains to be discussed; that is, the bearing of the results with histamine upon the effects following occlusion of different vascular areas. The optical curves show features in common, each presenting changes indicative of initial failure of normal dynamic relations. It might appear, therefore, that the occlusion effects were caused by metabolic products with histamine—like action formed during the periods of stasis. There seems little doubt that such products may contribute to the effects of occlusion, but that they are not the main cause is evidenced, I believe, by the relatively large amounts of histamine necessary to initiate failure changes and by the observation recorded above that during the periods of reëstablished circulation through the occluded part there are never indications of a dilating substance being carried into the general circulation.

SUMMARY

1. Alternate periods of occlusion and release of the circulation of three extremities, when continued for about 4 hours, produce changes in the optical pressure curve comparable to those shown in initial circulatory failure. There appears to be little difference whether the occlusion is produced by *a*, ligating all of the limb structures except the main arterial supply; or *b*, clamping the principal venous channels from these organs.

2. Clamping the inferior cava anterior to the renal veins shows indications in the pressure curve of cardiac embarrassment from diminished venous return. Two 30-minute periods of occlusion of this vein interrupted by 5-minute intervals of reëstablished flow produce

marked changes in the dynamic relations. By lessening the vascular area blocked, the time of appearance of these changes is somewhat delayed.

3. Blocking the venous return from a visceral area brings on a failure type of pressure curve much sooner than occlusion of a peripheral area of comparable vascularity.

4. The observations of a slow, progressive development of the occlusion effects of peripheral structures, the rapid onset with little evidence of progressive development for visceral areas, and the character of the changes portrayed in the pressure records, support the interpretation that the cause is essentially one of mechanical distention of the blood-bed in the occluded parts.

5. The optical pressure curves during the blood pressure change from a small dose of histamine show a sudden decrease in the peripheral resistance with a greatly diminished arterial distention, and an immediate recovery of peripheral tone. Injections of 0.04 to 0.05 mgm. repeated at 10-minute intervals produce after many such doses a small degree of decrease in peripheral resistance. Larger doses serve to accentuate these initial effects of the periodic dilatations.

6. Adrenalin augments the failure changes initiated by the repeated histamine injections.

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THE ARTIFICIAL PRODUCTION OF MONSTERS DEMONSTRATING LOCALIZED DEFECTS AS THE RESULT OF INJURY FROM X-RAYS

W. M. BALDWIN

From the Union University (Albany) Medical College

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In a recent paper (1919)[1] the author has detailed the results of a series of experiments upon frogs' ova where, by the use of X-rays generally applied to the surface of the ovum, a uniform defect was produced in the embryos. Inasmuch as a fixed quantity of energy was concentrated upon the ovum in the early stages of its development, practically all of the embryos presented the same gross and microscopic structural defects. In this present series of experiments, however, the X-ray energy was permitted to act upon a relatively small amount of the egg substance through the utilization of a perforated lead screen interposed between the tube and the specimen. The energy was derived from a large Coolidge tube which carried a milliamperage of 50 at 50 kilovolts. The interposed lead diaphragm measured about 24 mm. in thickness, through which a hole 0.3 mm. in diameter had been bored. Fertilized eggs of the frog, in a developmental stage no later than the two-celled, were placed behind the hole in the diaphragm in line with the center of the target of the tube and at a distance of 22.5 cm. from it. By this arrangement a maximum effect of X-ray energy upon a relatively small amount of the protoplasmic mass of the egg was brought about. An effort was made to so place the eggs that the energy entered at a point in the animal hemisphere and emerged at a corresponding point in the vegetable hemisphere. A columnar mass of protoplasm, 0.3 mm. in diameter, extending from the upper to the lower surface of the 1.7 mm. ovum, was subjected thereby to a maximum amount of direct radiant energy. In each instance an exposure varying from two to four minutes was

[1] The artificial production of monsters conforming to a definite type by means of X-Rays. Anat. Rec., xvii, no. 3, November, 1919. A bibliography list is appended.

made. The eggs were then transferred to a specimen jar containing 1000 cc. of tap water and permitted to develop at room temperature, the water being changed frequently.

It was ascertained after repeated experimentation first, that the amount of X-ray energy passing through the thickness of the 24 mm. screen at the distance given, and under the conditions of exposure mentioned, exercised no injurious effect whatever upon normal ova. Likewise, it was demonstrated that the secondary radiation arising from the edges of the hole in the diaphragm was so small in amount as to be a negligible factor. The effects recorded in these experiments were owing apparently, therefore, to the direct action of the X-ray energy upon the protoplasm of the egg using this term in its broadest sense, in the restricted region mentioned.

A study of the external features of the developed embryos reveals but little in comparison with the microscopic findings. In some instances it may be detected that the development of the eye or of the ear or both upon the same side is defective. Occasionally, as well, the gill tuft upon one side is absent. Furthermore, a lateral curvature of the body at the junction of the head with the trunk or at the level of the trunk, or at the level of the junction of the trunk with the tail may be observed, but this curvature is no greater than that which occurs with many normal tadpoles which are fixed together in large numbers. Some show an asymmetrical position of the dorsal fin at the level of several adjacent segments of the trunk. On the other hand, the color of the epidermis is normal throughout. The length, breadth and thickness of the tadpoles have not suffered to any appreciable degree over the controls.

A study of the serial sections of the tadpoles demonstrates, however, a peculiarly abnormal condition of the anlagen of several segments of the trunk and neck regions. As the sections are traced in sequence from the head to the tail, at a level beginning ordinarily at the optic chiasm and extending caudally to the region of the stomach, a marked difference is to be observed when the corresponding portions of the embryo to the right and to the left of the notochord are compared. Upon first glance the impression gained is that of unusual asymmetry of the embryo in which the neural tube, the notochord and the enteron have left their customary median position and migrated together toward one or the other side. The space ordinarily occupied by the mesodermal tissue and its derivatives is considerably smaller in area upon the side toward which the notochord has migrated than upon the

opposite side. At such levels the neural tube and the notochord, while occupying the real median plane of the embryo so far as anlagen are concerned, actually lie nearer one lateral trunk surface than the other by reason chiefly of the above mentioned inequality of development of the mesodermal derivatives. This inequality of mass distribution is the result of a retardation of development of certain consecutive hemisegments of the embryos. In no instance is this phenomenon restricted to one segment. Most often it involves those located immediately caudal to the point of origin of the optic stalk from the brain, and usually the defect extends as far caudally as the stomach.

A close microscopic examination of the cells in this defective area demonstrates the point that the various organ anlagen are not absent, but the processes of differentiation in the cells constituting them have been so greatly checked by the X-ray energy in contrast with those of the corresponding anlagen of the opposite half, that they present an appearance of the greatest degree of developmental retardation. The individual cells present certain features of both cytoplasm and of nucleus, moreover, which are unmistakable indications of deviations from the normal morphological characters of these cells.

The abrupt shifting of the neural tube, of the notochord and of the enteron from their normal median position in the mass of the embryo gives rise to the appearance of a lateral flexure of the trunk at the level of injury. In the caudal portion of the affected area a similarly abrupt reverse flexure brings these structures back to their normal median position. A singular feature of the abnormal embryos consists in the absence of any indication whatever of inflammatory reaction to injury in the affected area. Similarly the absence of extruded cells and of protoplasmic degenerations, involving either nucleus or cell body, may be interpreted as indicating a type of injury not of the most severe grade, as that noted by the author in his previous paper.

The cytoplasm of the cells in the affected area possesses a large, more-coarsely granular appearance than is normal. Pigment granules which, in the later stages of development, are more completely restricted to the epidermal cells, are to be observed in those cells the substance of which lay in the path of the rays through the ovum. Their presence in numbers enables one to mark out readily the direction of this path in the sectioned embryo. The nucleus of these cells presents, in the main, two general departures from normal; first, the chromatin is clumped either into large, deeply-staining masses restricted for the

most part to the periphery of the nucleus, leaving the center in contrast comparatively clear; or, secondly, the chromatin is more finely granular, more uniformly distributed, but invariably much less intensely staining than normal. It might be inferred that these two features represent different degrees of the same type of injury. There is no difficulty encountered, however, in readily differentiating between normal and rayed cells through these features. Furthermore, the contrasted appearance of these cells to normal cells in these partly-rayed specimens is all the more readily appreciated since both types may be seen in the same microscopic field.

In the previous paper on type embryos, the presence of nuclear and of protoplasmic detritus in the body cavities, such as the enteron, neurocele, optic vesicle, etc., was interpreted, as had been done by Hertwig previously with radium embryos, as a certain indication of such a degree of severe injury as to eliminate these cells from participation in the subsequent developmental stages of the embryo. Such features in this present series are, however, as was mentioned above, entirely absent. The injury produced by the rays was not sufficiently intense, apparently, to destroy the vitality of any of the cells. There ensued, on the contrary, a temporary suspension of differentiation followed by a lengthened tempo in the developmental rate of these affected cells. The absence of a reactive inflammation is, at present, difficult of interpretation unless we assume that possibly the damage wrought by the rays has been wholly intracellular.

As the different systems of the embryo are taken up in order and studied through the serial sections, several facts assume significant importance dependent upon the degree of reaction to X-ray influence. The ectoderm in the areas not in the path of the X-rays appears normal in thickness and in the disposition of its cells. Where the pencil of rays has passed through the embryo, however, those ectodermal cells in line with it depart from the normal in structure most markedly. In this area the entire thickness of the ectoderm in general is markedly increased. The individual cells are larger and the nuclei more deeply staining. The cell borders and the nuclear outlines are, however, distinct. This increase in thickness is, however, not uniform throughout the area affected, there being produced as a result an unevenness in the external surface of the ectoderm which amounts to a corrugation or wrinkling of this layer without involving, however, the basement membrane. In many instances the ridges so produced are hollowed out through the presence of channels which run a greater or less dis-

tance in a direction generally parallel to the underlying surface of the ectoderm. This reaction to X-ray influence resembles strongly that referred to by the author in his previous paper and noted as well by Hertwig in his study of radium embryos. There is no evidence of actual death or of desquamation of any of the ectodermal cells, neither are there indications of inflammatory reaction. In no specimen has the thickness of the layer been reduced.

As the brain and spinal cord are followed through the sections, there is to be noted at the level of injury a marked dwarfing of the brain vesicle upon the affected side. What appears to be a compensatory hypertrophy of the vesicle wall upon the unaffected side has resulted in a shifting of the median ventral furrow in the floor of the neurocele toward the affected side. This accentuates the appearance of asymmetry. The neuroblasts upon the unrayed side are normal in size, in shape and in the appearance of their nuclear and cytoplasmic material. Stratification ordinarily has progressed to such a degree that the external fiber layer may be distinctly differentiated from the nerve-cell layer. Upon the affected half of the vesicle there is a remarkable dwarfing of the neuroblast layer. The size of the individual cells is remarkably reduced, the nucleus is small and but faintly staining, and the protoplasm is but slightly granular. The sectional cross-area of the cell-mass constituting the floor of the vesicle is less than one-half that of the unaffected side. The dorsal median ridge in the roof of the brain occupies, however, its normal position. But very few bits of extruded nuclear or protoplasmic material are to be found in the neurocele. As the tube is traced cephalically or caudally, it is found to make a sharp, almost right-angled flexure away from the affected hemisegmental area, this flexure being so sharp when seen in the cross-sections as to appear as almost a longitudinal section. The purpose of these bends is naturally to bring the tube back into its normal median position in the unaffected segments of the body.

Dependent upon the level of the segments involved, either the optic vesicle or the otic vesicle, or both, upon the same side may be involved in the injury. Where the former has been encountered by the X-rays the cells constituting the optic stalk and the vesicle demonstrate the same abnormal features of nuclear and of protoplasmic structure as those presented by the neuroblasts upon the affected side of the brain. Stratification of the lining cells is absent; the lens is ordinarily not formed. These same features may be detected as well when the otic vesicle is involved in the injury. Where, however, a greater amount

of X-ray energy has acted upon these two organs, they are then entirely absent. The optic vesicle may be reduced to a mere stump attached to the ventral surface of the brain. There is no indication given through the thickening of the ectoderm of the formation of a lens. As was noted in the brain cavity, there is but little indication of the presence of extruded cells or of nuclei in these two vesicles.

The shifting of the notochord from its normal position toward the affected side is brought about by the same abrupt angularity of flexure as was noted in the neural tube. By reason, however, of a greater degree of migration, its normal ventral relationship to the tube is somewhat altered. There is nothing, however, to be noted as abnormal either in the appearance of its cells or in their arrangement.

Since the injury to these embryos is restricted to the neck and cephalic segments, the only portion of the enteron demonstrating abnormalities is the pharynx. In those segments where the injury has been severe but half of the pharyngeal wall is present. No effort has been made by the organism apparently to remedy the defect through the completion of the defective half of the tube-wall. The lining cells upon the affected half remain small, spherular and isolated. The number of them is greatly reduced and an orderly stratified grouping is completely absent. The general arrangement of these cells corresponds to that found in a very early stage of development. Differentiation of these cells, as was true also of the neuroblasts and of the cells of the optic vesicle, has been practically completely suspended. Upon the unaffected side, however, the pharyngeal wall is distinctly clothed by well-differentiated cells corresponding to the period of development. The contrast between these two halves of the pharyngeal wall is one of the most striking features presented by the embryos.

Similarly, the pronephros in the affected area is reduced greatly in the number of its tubules and in their size. The individual cells lining the walls of these tubules present features corresponding to those seen in the affected half of the pharynx, i.e., irregularity in arrangement together with spherulation and isolation of the cellular elements.

Cephalic to and caudal to the affected area the muscle segments are normally symmetrical in point of differentiation and of growth. In the affected segments, however, the myoblasts may be sharply contrasted from the standpoint of differentiation. The pigmentation of these cells, present normally only in early stages of development, is retained and, as well, the spherular, isolated, non-differentiated characters of the cells are distinctive. Upon the unaffected side the myo-

fibrillae may be readily distinguished. The myoblasts, which at this stage are elongated, have assumed their definitive arrangement with respect to the long axis of the embryo. These cells are sharply contrasted developmentally with those of the affected side. The cross-sectional area of the affected half of any myomere approaches ordinarily but less than half that of the unaffected side.

But few or no abnormal features may be discerned either in the heart or the pericardium. The great vessels passing through the rayed segments, on the other hand, demonstrate certain abnormal features. Most prominent among these is the retarded, embryonic condition of the lining endothelial cells and the undifferentiated condition of the tunics of the vessel walls. In only the largest of these have they made their appearance. Moreover the smaller blood vessels are entirely absent. The cellular elements which should assist in the formation of them remain small, spherular and isolated. There are no coördinated attempts at organization. As a direct result of these defects the gill tufts upon the affected side, normally dependent for their size chiefly upon the presence of these blood vessels, owing to their almost complete absence are markedly dwarfed or completely absent.

There is a great difference in size between the mesenchymal mass upon the affected half when compared with that upon the unaffected half of the same segments. That upon the affected half is much smaller owing to the defective size, shape and number of its cellular elements. The affected cells retain their earlier, embryological, spherular features. Isolation of the individual cells is most marked. The absence of mitotic figures in these cells is characteristic. Development of them has been strongly inhibited. There is no indication, however, of an edematous condition which might be anticipated in view of the absence of small blood vessels, thereby bringing about a separation of these cells as was found to be the case where the heart was markedly affected when the whole ovum was rayed. Microscopic study of these mesenchymal cells shows a lack of features of specialization in their protoplasm.

It is significant that evidences of reparative reaction to the injury sustained by the cells are entirely wanting. Assuming that the position of the neural tube and of the notochord indicates the median plane of the body, the cross-sectional area of the affected hemi-segments is reduced to less than half that of the normal segments. The several factors responsible for this condition are to be found, as was

noted above, in the undifferentiated condition and lack of growth of the myomeres, the pharynx, the blood vessels, the otic and optic vesicles and in the connective tissue-forming cells themselves.

These specimens demonstrate, therefore, the possibility of the production of localized injury by means of X-ray energy. This localization is sharply focused upon certain regions but involves all cells within the rayed area. This may be marked out most distinctly from the unaffected area. It is significant, in addition, that the injury received by the ovum at the twq-cell stage should be transmitted through so many cell generations without any greater manifest effort at restitution. Sharp localization of organ-forming substances at this early stage of development is brought out as well by the experiments. The nature of the change whether physical or chemical is of less importance from the embryological standpoint than is the demonstration of symmetrical distribution of these substances at so early a developmental stage. A consideration of the nature of the change wrought, whether an upset in the phase of an emulsion globule or a distinct oxidation or reduction of protein, lipin or carbohydrate is less striking than the phenomenon of the transmission and distribution to subsequent cell-generations of this same altered substance. Here again this feature is overshadowed by the manifest inability either of the cells or of the organism as a whole to rectify the damage done. The absence of cell extrusions, of protoplasmic and of nuclear detritus, all argue for an invariably uniform quantity of energy applied to the embryos, and so regulated as to avoid these evidences of more severe injury as were noted in the previous paper. The absence of blood-cellular features of inflammatory reaction may be referred to two possible sources, due either to a destruction of the anlagen of these elements but for which no definite evidence exists, however, or because of the intra-cellular and non-destructive character of the alteration produced.

THE RELATION OF THE EPINEPHRIN OUTPUT OF THE ADRENALS TO CHANGES IN THE RATE OF THE DENERVATED HEART

G. N. STEWART AND J. M. ROGOFF

From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University

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INTRODUCTION

It is well known that adrenalin causes acceleration of the heart after section of the vagi and excision of the stellate ganglia. v. Anrep (1) showed in the dog that stimulation of the peripheral end of the splanchnic nerve caused acceleration, associated with the peculiar features of the splanchnic blood pressure curve which Elliott (2) explains as due to the augmented epinephrin output known to be elicited by stimulation of that nerve. Pearlman and Vincent (3), working with the heart only partially isolated from the central nervous system by section of the vagi, have confirmed Elliott's interpretation of the peculiar form of the blood pressure curve caused by splanchnic stimulation and observed manifest augmentation of the heart. We have ourselves shown (in the cat) that the epinephrin given off at the normal average rate under the conditions of our experiments causes acceleration when the adrenal vein blood collected in a cava pocket for a minute or two is released. The acceleration begins a little time after release of the pocket, the length of the interval depending upon the state of the circulation, and is coincident or nearly so with the dilatation of the pupil if the superior cervical ganglion has been previously excised. The beginning of the dilatation has been seen to coincide approximately with the "dip" associated by Elliott with the action of the liberated epinephrin (4). Also when the splanchnic is stimulated with the adrenal veins open it can sometimes be seen that about the same time as the reactions of the eye (sensitized by removal of the superior cervical ganglion) appear, the denervated heart begins to become accelerated. From all this it seems clear that epinephrin

liberated from the adrenal in response to stimulation of the splanchnic plays a part in the acceleration of the heart. We shall show later on that the whole acceleration is not due to the epinephrin, but that other factors are involved, since a good acceleration, even as great as that obtained with intact adrenals, can be observed when they have been removed or when discharge of epinephrin has been prevented in other ways. It will be pointed out in discussing these results that, with such a reaction as acceleration of the heart, there is no inconsistency in attributing a share in the reaction to epinephrin and yet asserting that sometimes as great a maximum acceleration may be attained in its absence as when it is being given off. It is possible that the position of the maximum acceleration or of the beginning of the acceleration on the blood pressure curve may be shifted when epinephrin action is excluded. But however this may be, it will, we believe, be evident when our results have been displayed that it would be a very unpromising venture to attempt to found a method of estimating the rate of output of epinephrin upon such a reaction, even under conditions, as in splanchnic stimulation, in which it is known that an increase in the epinephrin output can contribute to it. Where adrenal blood is collected in a cava pocket and then released there is no question that the acceleration is produced by the epinephrin and practically by that alone, because the experiment has been so simplified that only one factor is acting, namely the admission into the circulation of the epinephrin-containing blood. But even here the reaction is of such a character that it could hardly lend itself to anything like an exact assay of the epinephrin in the blood from the pocket.

Nevertheless in a recent paper Cannon (5) has described experiments on cats in which, from the acceleration of the so-called denervated heart caused by stimulation of the central end of the sciatic nerve, by asphyxia and by emotional excitement, he professes to prove that these conditions produce a marked increase in the rate of output of epinephrin from the adrenals. Apparently admitting that the catheter method (6) is a difficult one to obtain positive results with, he introduces this as a relatively simple method which can be carried out by "any competent experimenter," and he states that the results of these experiments confirm "in every particular" his previous conclusions as to the influence of emotional excitement, asphyxia and sensory stimulation upon the adrenal secretion. We have never quarreled with the catheter method because of its difficulty, but because it cannot yield the data necessary to determine the rate of output of epinephrin or to

measure the changes in that rate. We shall take another occasion to point out again the reasons why we cannot accept Cannon's conclusions based on experiments with the catheter method. The technique of obtaining the cava blood is surely not beyond the reach of "any competent experimenter" in physiology, and the assaying of the epinephrin in the blood would be easy if such concentrations existed there as, by implication, we must conclude that Cannon assumed to exist during or after the action of the factors studied by him.

The denervated heart reaction now adopted by Cannon "as an indicator of adrenal secretion" is also easy enough to carry out. But it labors under even more serious defects, when employed as a quantitative method of measuring the epinephrin output and of estimating changes in the rate of output, than does the catheter method. For in the latter an attempt was at any rate made to obtain blood containing a portion of the epinephrin given off by the adrenals and to test it by a method of bio-assay which, if properly applied, does permit the epinephrin concentration in the sample of blood to be estimated. When Doctor Cannon stimulates the central end of the sciatic numerous reflex effects may be caused, the consequences of which upon the rate of the heart cannot be easily controlled. Among these the vasomotor reflex changes are conspicuous, and it is obvious that in this way great variations may be produced in the pressure in the cavities of the heart, the rate of blood flow through the coronary system and, therefore, the amount of epinephrin passing through the coronaries, any of which may lead to an acceleration of the heart beat without any change having occurred in the rate of output of epinephrin.

Cannon, however, states that after removal of the adrenals or their ligation *en masse* or after removal of one adrenal and section of the splanchnic of the other side, acceleration of the heart is no longer caused by sciatic stimulation. In one experiment he obtained some acceleration after division of both splanchnics in the thorax, but much less than before. He concludes that the acceleration following sciatic stimulation is due entirely to a reflexly increased output of epinephrin. And obviously assuming that no other factors are involved, he states that "comparisons of the increased rate due to sciatic stimulation with effects of adrenalin (quantitated as base) injected intravenously indicate that the range of reflex adrenal secretion lies between 0.001 and 0.005 mgm. per kgm. per minute, i.e., from 5 to 25 times the amount regarded by Stewart and Rogoff as the normal output." In view of our own results proving conclusively that epinephrin, if a factor, cannot

be the sole factor in the heart reaction elicited by stimulation of the sciatic, it would scarcely be worth while to spend much time in examining such data. But it may be pointed out that Cannon does not state by what control experiments he has established a quantitative relation between the *maximum* acceleration reached and the dose of adrenalin, and not, for instance, between the dose of adrenalin and the duration of the acceleration or the total surplus number of beats in the period of acceleration. Also if such data are to have real quantitative value the adrenalin ought to be administered while the blood pressure and therefore the coronary blood flow are increased to approximately the same extent as during stimulation of the sciatic.

As regards Cannon's statement that the reaction is abolished by removal of the adrenals, it must be noted that, if it were granted that the only change caused by removal of the adrenals or section of the splanchnics is the suppression of the epinephrin output, his result would simply show that the acceleration previously obtained had been due essentially to epinephrin. It would not show that any increase had occurred in the rate of output, unless it were demonstrated that a redistribution of the blood, due to the vascular reactions evoked by the stimulation and necessarily associated with the passage through the coronary circulation of an increased proportion of the epinephrin already being given off, was insufficient to account for the reaction.

But it cannot be granted that the operations practised to eliminate the epinephrin output have no other consequences. It is astonishing with what indifference both splanchnics are cut merely in order to interfere with the epinephrin output, as if all or most of the splanchnic fibers innervated the adrenals. The same is true of the removal of the adrenals. Some writers seem to assume that it is practically impossible to injure any important nerves when the adrenals are removed or tied off and that all the consequences which follow their removal are necessarily due to the loss of epinephrin. Gley and Quinquaud (7) have pointed out that the opposite is the case. In the dog according to them it is practically impossible to remove the adrenals without severely injuring the splanchnics. They say, however, that with their large experience they have succeeded in operating on the dog also, so as to eliminate the adrenals without injury to the splanchnics. Pearlman and Vincent (3) take exception to Gley's statement that the difference in the splanchnic blood pressure curve observed by v. Anrep (1) in dogs after and before removal of the adrenals is due to injury to nerve fibers, and no doubt in Vincent's hands the operation is as little harmful as it

is possible for it to be. If the operation can be more easily done in the cat, it still needs care and experience to reduce this cause of error to a minimum, particularly in the case of the right adrenal, the ligation of which is liable to injure the splanchnic. For this and other reasons the removal of the adrenals, as frequently done in acute experiments, may be attended with a marked drop in the blood pressure not related to loss of function of the glands. It must be remembered that in experiments on the denervated heart, the adrenals are removed after a considerable preliminary operation and the extrinsic regulatory nerves of the heart are eliminated. This may make it more difficult to excise or tie off the adrenals without a fall of blood pressure than in experiments on normal animals, such as those of Young and Lehman (8), of Hoskins and McClure (9) and of Bazett (10). The diminution in the heart rate remarked by Cannon, and apparently attributed by him entirely to the loss of epinephrin, is according to our observations not unrelated to the fall of pressure, although since there is reason to believe that the epinephrin liberated at the ordinary rate may affect the heart, some part of the slowing may be due to loss of epinephrin. Cannon has not given any data by which one can judge how great the change of blood pressure was in his experiments, but as he injected gum salt solution in one of them the fall may be assumed to have been sometimes considerable. Now, whatever interpretation one chooses to put upon the heart acceleration produced by sciatic stimulation, a reflex or more than one reflex action must be essentially involved in it. Any operation which impairs the conductivity of the reflex arcs must, therefore, tend to diminish or abolish the effect. And if a negative result obtained after removal of a certain organ is attributed solely to the specific effect which the operator intended to produce, and not at all to the general effects which he did produce, then, of course, positive results elicited before the operation in any way whatever will seem to depend entirely upon the specific activity of the organ removed.

In what has been said above we desire to state distinctly that we do not imply that Doctor Cannon did not remove the adrenals skilfully, and with full knowledge of the importance for his control experiment of injuring the nerves in the vicinity as little as possible and of maintaining the animal in a good general condition. All we know is that we obtained positive results after elimination of the adrenals where he obtained completely negative results. It is clearly the positive results which are significant for the decision of the question of the relation of

the epinephrin output to the heart acceleration caused by sciatic stimulation and not the negative ones.

Our experiments were all made on cats. The greater number of them were performed two years ago. Only a brief notice of a portion of them has been published (11). They were mentioned, so far as could be done in the few minutes allotted to us, in the discussion of Doctor Cannon's paper at the meeting of the American Physiological Society last December. We studied the effect upon the acceleration produced by stimulation of sensory nerves of eliminating the epinephrin secretion:

a. By clipping the adrenal veins, either with or without simultaneous ligation of the renal vessels.

b. By removing one adrenal (the right) and denervating the other and allowing the animal to recover completely from the operation. In a number of these animals the denervated adrenal was also removed in the final experiment, and the heart reactions obtained before and after its removal compared.

c. By removing one adrenal (almost always the right) and allowing an interval for the animal to recover, before performing the experiment upon sensory nerve stimulation with removal of the other adrenal. In this way it was supposed that the condition of the animal after removal of the remaining adrenal would be better than if both were removed at the time of the experiment.

d. By removing both adrenals during the experiment on the heart reaction. In all cases in excising the glands every precaution was taken to avoid injury to nerves, by making a careful dissection between the capsule of the gland and the cortex, tying the vessels with fine ligatures.

EXPERIMENTS IN WHICH THE ADRENAL VEINS WERE CLIPPED OFF OR TIED

In principle this is the most satisfactory way of eliminating the epinephrin output, since no other organ than the adrenals is interfered with and there is no damage to important nerves and no injurious fall of blood pressure. The procedure has been extensively employed by Gley. v. Anrep (1) also used it in some of his experiments on splanchnic stimulation and satisfied himself that the effects attributed by him to epinephrin were not obtained when the suprarenal vein was clipped and the corresponding splanchnic stimulated. And Pearlman and Vincent (3) state that they have usually obtained quite satisfactory

results by simply clamping and unclamping the adrenal veins. Cannon takes exception to clipping because it does not eliminate the heart reaction to sciatic stimulation or asphyxia which he interprets as indicating increased epinephrin output, and he, therefore, assumes that there must be leakage of epinephrin by anastomotic venous channels. He quotes, for instance, an experiment in which before ligation of the adrenal veins asphyxia of a certain duration caused an acceleration in the heart rate of 40 beats. After ligation of the veins the acceleration was precisely the same. We should have thought the only possible interpretation of such a result would have been that epinephrin had nothing to do with the reaction, in the particular experiment, at any rate. For who will believe that after the adrenal veins were tied just as much epinephrin passed out of the adrenals by some difficult collateral path, in the minute for which asphyxia was maintained, as would have passed out by the adrenal veins plus these hypothetical anastomotic channels? And how can the heart acceleration be a quantitative reaction for epinephrin if blocking the adrenal veins does not at least diminish the reaction? When a reaction known to be caused by epinephrin is studied the result is quite different. For example, we sometimes observe a small dilatation of the pupil (after previous removal of the superior cervical ganglion) on stimulation of the peripheral end of a splanchnic nerve, with the corresponding or even with both adrenal veins clipped. But this is much smaller than the reaction obtained before or after by similar stimulation with the veins open, and usually on release of the clips there is an additional and greater reaction indicating that epinephrin had been pent up in the adrenal veins by the clips. We suggest that the reason why Cannon gets such positive results with the adrenal veins tied, and must have recourse to removal or ligation of the adrenals to obtain negative results, is that in tying the veins he does not inflict such injury as causes a general deterioration of the animal incompatible with a positive heart reaction, whereas in removing the adrenals he appears to do so.

In our experiments with clipping of the adrenal veins we compared the heart acceleration and rise of blood pressure caused by stimulation of the sciatic when the blood flow from the glands and, therefore, the epinephrin output were going on unhindered, with the acceleration and blood pressure rise obtained when the flow from one or both adrenals was obstructed. It was supposed that if the epinephrin is an important factor in the heart reaction, the reaction would be distinctly

smaller with the adrenal veins clipped off, that is to say, provided that the reaction can be used at all as a quantitative test for epinephrin. The protocols show that this expectation was not realized. If the epinephrin liberated from the adrenals is an appreciable factor, it is not easy to disentangle its influence from that of the other factors which can affect the heart rate. It must be remembered that even if it were clearly demonstrated that the acceleration caused by sciatic stimulation was diminished by interference with the output of epinephrin, the other potential factors, such as rise of blood pressure, not being interfered with, this would only prove that epinephrin takes a share in the reaction, not that its rate of output is increased by the stimulation.

The peripheral end of a splanchnic nerve was also stimulated with the corresponding, or both adrenal veins clipped or open, in order to compare the effect of a procedure which is known to increase the epinephrin output on the heart rate with the effect of sciatic stimulation.

It will be seen that it may be difficult to demonstrate, by comparing the maximum acceleration caused with the adrenal veins clipped and open, that the epinephrin undoubtedly liberated by stimulation of the splanchnic takes any sensible share in the heart reaction. However, this is rather an illustration of the deficiencies of such a reaction as a quantitative test for epinephrin than a proof that the epinephrin liberated with the adrenal veins open is without effect upon the rate of the heart. As already mentioned, when the epinephrin is allowed to accumulate in a cava pocket or even when the epinephrin pent up in the adrenal vessels by clipping of the adrenal veins is released a distinct acceleration is produced, and it may be assumed that the epinephrin coming steadily off from the adrenals, without being accumulated, will tend to exert a similar action, especially when the amount of epinephrin passing per unit of time through the coronaries, or its concentration, is abruptly increased by the vasomotor changes associated with splanchnic or sciatic stimulation. It seems, however, improbable that with such a reaction as acceleration of the heart the acceleration produced by simultaneous action of two factors, singly effective, should be the sum of the separate effects, whether the reaction is measured by the maximum acceleration attained, or by the duration of the acceleration or by the total surplus number of beats. It seems more likely that when the heart is keyed up to a certain point by the action of one factor, whether this acts upon a local accelerator mechanism or not, it will break loose, so to speak, from the relative stability of rate

imposed upon it by removal of its extrinsic nerves, and execute a run of quicker beats, the duration and maximum acceleration of which may have no simple relation to the absolute magnitude of the exciting influence, and which may not be greatly modified by a concomitantly acting influence, itself capable of independently producing a similar reaction. The results of these experiments are illustrated by some protocols. Where it is mentioned that a nerve was stimulated with a vein clipped, the clip was applied, unless otherwise stated, a few seconds before the stimulation and was removed as soon as the portion of the curve to be used for counting the heart rate had been completed. When it is simply mentioned that a nerve was stimulated, without any reference to clipping, it is implied that the veins were open. Under "rate" is always given the number of heart beats per minute; under "pressure" the blood pressure in millimeters of mercury.

Protocol. Cat 175; male; weight, 2.2 kgm.
11:30 a.m. Urethane, 5 gm. by stomach tube.
1:10–1:48 p.m. Cut vago-sympathetics and excised stellate ganglia;[1] prepared central end of left sciatic for stimulation.

		Rate
1:50 p.m.	Before stimulation of sciatic	188
	25 seconds after beginning stimulation	210
1:55 p.m.	Before stimulation of sciatic	187
	20 seconds after beginning stimulation	202
	40 seconds after beginning stimulation	212
	Now opened abdomen and purposely manipulated intestines	
2:03 p.m.	Before stimulation of sciatic	178
	30 seconds after beginning stimulation	212
2:10 p.m.	Before stimulation of sciatic	186
	30 seconds after beginning stimulation	214
2:14 p.m.	Before stimulation of sciatic; left adrenal vein clipped	184
	30 seconds after beginning stimulation	206
2:16 p.m.	Prepared peripheral end of left splanchnic in abdomen	
2:20 p.m.	Before stimulation of splanchnic; left adrenal vein clipped	184
	15 seconds after beginning stimulation	192
	30 seconds after beginning stimulation	208
	45 seconds after beginning stimulation	224

[1] With the cat in the supine position an incision is made in the axilla and a part of the second rib exposed by separation of overlying muscle. A portion of the rib anterior to the angle is carefully separated from its periosteum and resected. The lower part of the stellate ganglion is usually thus exposed and the excision of the ganglion easily completed without injury to the pleura.

In all the experiments, except when otherwise stated, the period of stimulation of the sciatic was 30 to 40 seconds. When the heart rate is given after a certain number of seconds from beginning of stimulation this means that a sufficient portion of the curve beginning at that point was counted. The corresponding blood pressure was that at the end of the portion counted.

2:26 p.m.	Before stimulation of splanchnic; left adrenal vein clipped	180
	15 seconds after beginning stimulation	196
	30 seconds after beginning stimulation	208
	45 seconds after beginning stimulation	220
	60 seconds after beginning stimulation	220
2:32 p.m.	Before stimulation of splanchnic	182
	During first 15 seconds of stimulation	180
	During second 15 seconds of stimulation	200
	During third 15 seconds of stimulation	204
	During fourth 15 seconds of stimulation	216
2:47 p.m.	Before stimulation of sciatic; both adrenal veins clipped	184
	During first 15 seconds of stimulation	188
	During second 15 seconds of stimulation	188
	During third 15 seconds of stimulation	200
	During fourth 15 seconds of stimulation	204
2:53 p.m.	Before stimulation of sciatic	176
	During first 15 seconds of stimulation	180
	During second 15 seconds of stimulation	196
	During third 15 seconds of stimulation	208
	During fourth 15 seconds of stimulation	216
3:05 p.m.	Before stimulation of sciatic; both adrenal veins clipped	188
	During first 15 seconds of stimulation	188
	During second 15 seconds of stimulation	196
	During third 15 seconds of stimulation	204
	During fourth 15 seconds of stimulation	204

		Rate	*Pressure*
3:45 p.m.	Before stimulation of sciatic	176	82
	24 seconds after beginning stimulation	203	140
3:50 p.m.	Before stimulation of splanchnic; left adrenal vein clipped	174	58
	28 seconds after beginning stimulation	215	166
3:57 p.m.	Before stimulation of splanchnic; both adrenal veins clipped	185	62
	24 seconds after beginning stimulation	206	131
	40 seconds after beginning stimulation	221	
4:03 p.m.	Before stimulation of sciatic	174	46
	12 seconds after beginning stimulation	197	118
	40 seconds after beginning stimulation	200	
4:10 p.m.	Before stimulation of sciatic; both adrenal veins clipped	189	52
	15 seconds after beginning stimulation	200	112
	30 seconds after beginning stimulation	204	
4:15 p.m.	Before stimulation of splanchnic	184	55
	20 seconds after beginning stimulation	209	130
	Excised left adrenal		
4:30 p.m.	Before stimulation of splanchnic	215	70
	22 seconds after beginning stimulation	229	90

Some of the curves from these experiments are used in the next paper to demonstrate that the acceleration of the heart on stimulation of the central end of the sciatic, contrary to Cannon's statement, is easily obtainable after the abdomen has been opened. The protocols themselves and other protocols coming later in the present paper also afford abundant evidence that the statement is baseless. In figure 1 portions of the blood pressure curve from cat 175 before and during stimulation of the left splanchnic (*A* and *B*, taken at 3:50 p.m.) are

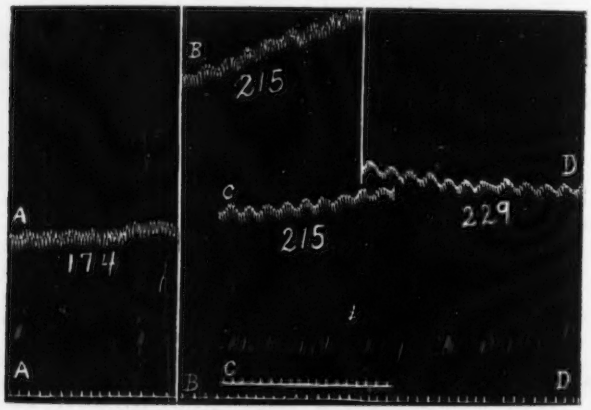

Fig. 1. Parts of blood pressure tracings from cat 175. *A*, before and *B*, a portion commencing 28 seconds after beginning of splanchnic stimulation with corresponding adrenal vein clipped; *C*, before and *D*, 22 seconds after beginning of splanchnic stimulation with corresponding adrenal excised. In all figures line of zero pressure corresponds with time trace; time in seconds; numbers above time trace represent heart rate per minute. Reduced to four-fifths.

reproduced, and corresponding portions after removal of the adrenal (*C* and *D*, taken at 4:30 p.m.). The maximum acceleration in *B* was 41 beats per minute; the maximum in *D*, 12 to 14 beats as compared with a maximum acceleration of 25 beats in the curve taken with the last splanchnic stimulation prior to removal of the adrenal. It is impossible to know how much, if any part, of the difference is due to the lack of epinephrin from the left adrenal when the curve *C D* was being written, since the stimulation was much less effective in raising the blood pressure, as shown in figure 2. The much reduced curves (black on white) are not intended for counting.

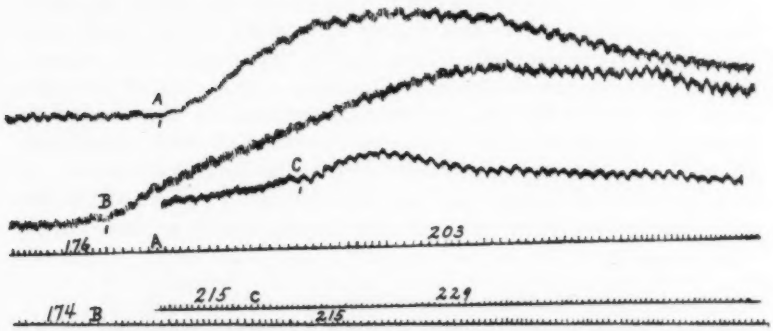

Fig. 2. Blood pressure curves from cat 175. *A*, sciatic stimulation; *B*, splanchnic stimulation with corresponding adrenal vein clipped; *C*, splanchnic stimulation with corresponding adrenal excised, 40 minutes after *B*. Reduced to one-half.

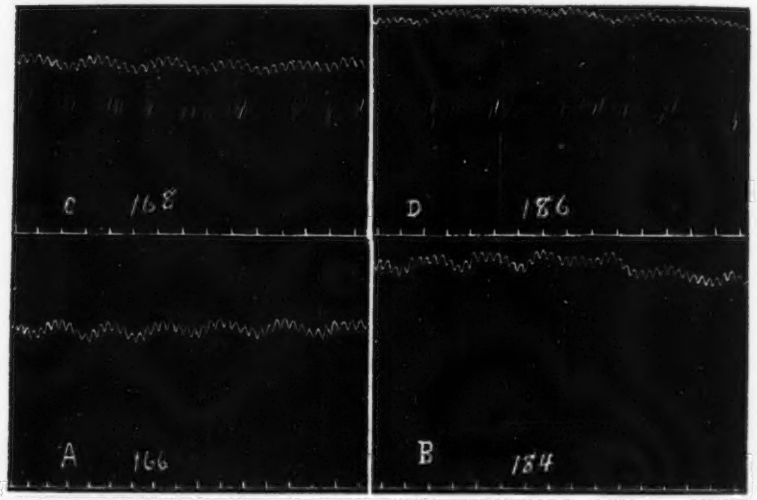

Fig. 3. Parts of blood pressure tracings from cat 237. *A*, before and *B*, a portion commencing 33 seconds after beginning of sciatic stimulation; *C*, before and *D*, 40 seconds after beginning of sciatic stimulation with both adrenal veins clipped. Reduced to three-fourths.

In figure 3 portions of the curve from cat 237 are given, *A* and *B* taken respectively before and during stimulation of the sciatic with the adrenal veins open and *C* and *D* with the adrenal veins clipped. The curves were practically parallel and the acceleration of the heart about the same in both (about 18 beats per minute), in the portions shown in the figure. The maximum acceleration with stimulation after clipping was 21, and without clipping 20 beats per minute. It may be remarked that not only had the abdomen been opened but the renal arteries and veins had been tied 40 minutes before these curves were obtained.

Protocol. Cat 177; male; weight, 3.6 kgm.

2:05 p.m. Under urethane (6 gm.) cut vago-sympathetics; excised stellate ganglia and prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
2:25 p.m.	Before sciatic stimulation	185	132
	20 seconds after beginning stimulation	228	154
2:30 p.m.	Opened abdomen; prepared peripheral end of left splanchnic		
2:32 p.m.	Before sciatic stimulation	173	105
	24 seconds after beginning stimulation	200	126
	49 seconds after beginning stimulation	216	
2:35 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	167	108
	12 seconds after beginning stimulation	191	
	24 seconds after beginning stimulation	215	140
	45 seconds after beginning stimulation	211	110
2:40 p.m.	Before stimulation of sciatic; both adrenal veins clipped	181	80
	23 seconds after beginning stimulation	190	
	37 seconds after beginning stimulation	197	106
2:45 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	171	90
	17 seconds after beginning stimulation	194	
	22 seconds after beginning stimulation	206	124
2:50 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped	191	100
	20 seconds after beginning stimulation	213	128
2:55 p.m.	Before stimulation of sciatic; left adrenal vein clipped	184	94
	28 seconds after beginning of stimulation	192	107
3:00 p.m.	Before stimulation of sciatic; both adrenal veins clipped	167	78
	23 seconds after beginning stimulation	173	98
3:10 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	160	74
	18 seconds after beginning stimulation	194	92

3:15 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped	160	82
	23 seconds after beginning stimulation	167	
	42 seconds after beginning stimulation	185	
	55 seconds after beginning stimulation	200	106
3:20 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	165	72
	20 seconds after beginning stimulation	183	82
3:30 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped	166	68
	25 seconds after beginning stimulation	186	86
3:35 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	173	76
	20 seconds after beginning stimulation	180	90
4:10 p.m.	Before stimulation of peripheral end of right splanchnic in thorax, with left adrenal vein clipped	170	30
	15 seconds after beginning stimulation	205	78

In cat 177, in which a good acceleration of the heart was obtained with sciatic stimulation after opening the abdomen and section of one splanchnic nerve, the reaction from the sciatic after repeated splanchnic and sciatic stimulations and repeated clipping of the adrenal veins was distinctly diminished toward the end of the experiment. Stimulation of the peripheral end of the splanchnic continued to give a good acceleration of the heart when the reaction, as reflexly elicited by stimulation of the central end of the sciatic, together with the reflex rise of blood pressure, was being exhausted.

In comparing the acceleration accompanying a given rise of blood pressure caused by sciatic, with that accompanying a similar rise of pressure caused by splanchnic stimulation it generally appeared that the splanchnic acceleration was the greater. This, of course, would be consistent with the view that the epinephrin secretion in response to direct splanchnic stimulation may play a substantial part in the reaction. It would appear probable that any share of the epinephrin in the acceleration both when the output is increased, as by splanchnic stimulation, and when, without an actual increase in the output, more epinephrin is sent through the coronary circulation in response to a rise of blood pressure caused by sensory nerve stimulation, must vary with the state of the heart and may, therefore, be expected to vary in different animals and in the same animal at different periods of an experiment.

Protocol. Cat 179; male; weight, 2.2 kgm. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
4:00 p.m.	Before sciatic stimulation	169	80
	25 seconds after beginning stimulation	210	130
4:05 p.m.	Prepared peripheral end of left splanchnic (extraperitoneally)		
4:10 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	168	70
	20 seconds after beginning stimulation	182	94
4:13 p.m.	Before sciatic stimulation	173	81
	32 seconds after beginning stimulation	204	113
4:17 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	177	73
	20 seconds after beginning stimulation	180	98
4:20 p.m.	Before stimulation of left splanchnic	170	80
	24 seconds after beginning stimulation	183	109
4:25 p.m.	Before stimulation of sciatic; left adrenal vein clipped	170	76
	23 seconds after beginning stimulation	180	106
4:28 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	170	71
	23 seconds after beginning stimulation	180	100
4:30 p.m.	Opened abdomen; manipulated intestines		
4:32 p.m.	Before sciatic stimulation	171	66
	25 seconds after beginning stimulation	196	102
4:35 p.m.	Before stimulation of sciatic; both adrenal veins clipped	173	64
	20 seconds after beginning stimulation	192	95
4:40 p.m.	Before stimulation of left splanchnic with both adrenal veins clipped	173	70
	21 seconds after beginning stimulation	179	103
4:45 p.m.	Before stimulation of splanchnic	171	62
	25 seconds after beginning stimulation	179	90
4:48 p.m.	Cut right splanchnic		
4:50 p.m.	Before sciatic stimulation	160	44
	23 seconds after beginning stimulation	161	56

The protocol of cat 179 shows again quite clearly good heart reactions with sciatic stimulation after opening the abdomen and section of one splanchnic. The practical disappearance of the reaction after section of the remaining splanchnic, when the blood pressure fell to 44 mm. of mercury is well shown. But is it likely that this disappearance is due solely to the suppression of the epinephrin secretion when a little earlier in the experiment a decided reaction was obtained by stimulation of the sciatic with both adrenal veins clipped and after section of the left splanchnic? Here the blood pressure reaction was a

good one, the pressure rising from 64 to 95 mm. of mercury. But after the section of the second splanchnic only a trifling rise of pressure was caused by sciatic stimulation. The absence of any substantial rise of pressure and the depressing influence of the lowered blood pressure on the paths for any other reflexes than the vascular reflexes which may be concerned in the reaction offer a more probable explanation.

In a number of cats one superior cervical ganglion (the left) had been previously excised, so that it was possible to compare the acceleration of the heart caused by stimulation of the sciatic or splanchnic with the eye reactions. The following is a typical protocol.

Protocol. Cat 190; female; weight, 2.31 kgm. Left superior cervical ganglion excised 21 days previously. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
1:05 p.m.	Before stimulation of sciatic[2]	253	123
	35 seconds after beginning stimulation	259	164
1:10 p.m.	Before sciatic stimulation[2]	229	
	15 seconds after beginning stimulation	237	
1:15 p.m.	Prepared peripheral end of left splanchnic (extraperitoneally		
1:35 p.m.	Before stimulation of left splanchnic	231	80
	10 seconds after beginning stimulation	268	155
	Very good pupil and nictitating reactions in 9 seconds		
1:40 p.m.	Before stimulation of left splanchnic with left adrenal vein clipped	223	64
	20 seconds after beginning stimulation	280	150
	Small pupil and nictitating reactions in 18.4 seconds		
1:50 p.m.	Before stimulation of left splanchnic with left adrenal vein clipped	227	70
	20 seconds after beginning stimulation	263	
	48 seconds after beginning stimulation	278	130
	Very small eye reactions in 24.8 seconds		
2:15 p.m.	Excised left adrenal		
2:20 p.m.	Before stimulation of left splanchnic	219	72
	25 seconds after beginning stimulation	227	100
	Doubtful, if any eye reactions		

[2] Both pupils dilate instantaneously, the left becoming much wider than the right, both returning to previous state very soon after stimulation of the sciatic is stopped. With a prolonged or strong stimulation the left nictitating may slowly retract. This reaction is distinctly different from that obtained with splanchnic stimulation (when the adrenal veins are free) which affects only the denervated eye and occurs after a distinct latent period.

2:30 p.m.	Before stimulation of sciatic	223	80
	22 seconds after beginning stimulation	262	168
2:35 p.m.	Opened abdomen; prepared peripheral end of right splanchnic		
2:40 p.m.	Before stimulation of right splanchnic	210	45
	20 seconds after beginning stimulation	285	105
2:55 p.m.	Before clipping aorta just above diaphragm	249	48
	After clipping aorta just above diaphragm	260	146

It is to be remarked that when the left splanchnic was stimulated with the left adrenal vein clipped, the eye reactions were not completely abolished but were much diminished and appeared after a much longer delay than when the splanchnic was stimulated with the vein open. Complete loss of the eye reactions on stimulating the peripheral end of a splanchnic nerve may be seen when the corresponding adrenal vein has been clipped off, although good reactions were being obtained with the vein open, or the result may be what was found in cat 190. This result is compatible with the view that some epinephrin may find its way into the blood stream by a collateral route, as suggested by Cow (12) (the renal vessels were not tied in this experiment*) but may also be due solely to a change in the concentration or quantity of epinephrin from the other adrenal passing through the coronary circulation, associated with the vasomotor effects of the splanchnic stimulation. The observation that in this experiment after excision of the left adrenal stimulation of the left splanchnic produced only a doubtful, if any, pupil reaction does not enable us to decide against the latter view. For the vasomotor effect (rise of blood pressure) and the heart acceleration were also much reduced, possibly owing to some injury to the splanchnic in removal of the adrenal. The fact that the eye reactions, while still present, were greatly diminished by the clipping of the adrenal vein and that it took a much greater time for them to appear while the acceleration of the heart was not at all diminished, shows clearly that the latter reaction cannot be a quantitative test for epinephrin. With sciatic stimulation the pupil reaction is complicated by the fact that both pupils dilate immediately after stimulation, of course through the nervous system. Although the sensitized pupil widens more than the other, this reaction is different from the typical epinephrin reaction yielded, for instance, by splanchnic stimulation. The return of the pupils to their previous size on stoppage of the stim-

*In making a cava pocket we generally tie the renal vein at the hilus and also at its entrance into the cava.

ulation begins at once and is accomplished more quickly after sciatic stimulation than when a true epinephrin reaction has been induced by splanchnic stimulation. Further, the pupil reaction elicited by stimulation of the sciatic after interference with the epinephrin output by removal of one adrenal and section of the nerves of the other, or after removal of both adrenals, has the same characters as that seen with intact adrenals. A nictitating reaction was not obtained with sciatic stimulations which caused marked acceleration of the heart. Only with quite strong stimulation of the sciatic was there any retraction of the nictitating. This also is different from the genuine epinephrin eye reactions. The observations on the eye reactions, then, are inconsistent with the idea that stimulation of the sciatic causes a marked increase in the output of epinephrin, which in its turn causes the observed acceleration of the heart. It may further be asked how an acceleration (see protocol of cat 190) caused by sciatic stimulation, which is much smaller with both splanchnics intact at the beginning of the experiment than later on when one splanchnic has been divided and the corresponding adrenal removed, can be considered a quantitative reaction for the rate of output of epinephrin. It will be seen in another section of the paper that it is common, or indeed the rule, to obtain as large a reaction on stimulation of the sciatic in animals from which one adrenal has been removed and the animal allowed to recover as in animals with both adrenals intact.

Another cat (191) from which one superior cervical ganglion had been previously removed yielded results so similar to those of cat 190 that the protocol need not be reproduced. The only difference was that sciatic stimulation, as usual, gave a good acceleration at the beginning of the experiment, from 240 to a maximum of 295 beats per minute, the blood pressure rising from 124 to 182 mm. of mercury. With a second stimulation of the sciatic the heart rate increased from 256 to 275, the blood pressure rising from 112 to 150 mm. The eye reactions were the same as in cat 190. The abdomen was now opened and the peripheral end of the left splanchnic stimulated. The pulse rate rose from 243 to 270 beats, the blood pressure from 90 to 144 mm. of mercury. Excellent pupil and nictitating reactions were obtained in 9 seconds. The left adrenal vein was then clipped and the left splanchnic again stimulated. The heart rate increased from 237 to 262 beats per minute and the blood pressure from 110 to 150 mm. of mercury. Small pupil and nictitating reactions were observed in 20.4 seconds.

Protocol. Cat 195; female; weight, 2.4 kgm. Left superior cervical ganglion excised 14 days previously. Under urethane (5.5 gm.) prepared peripheral end of left splanchnic for stimulation in abdomen, tied lumbar veins just before they cross the adrenals; prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
2:55 p.m.	Before stimulation of left splanchnic	202	78
	12 seconds after beginning stimulation	216	96
	Very good pupil and nictitating reactions in 12.8 seconds		
3:00 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	186	80
	19 seconds after beginning stimulation	211	108
	No eye reactions		
3:05 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped	178	71
	14 seconds after beginning stimulation	181	78
3:20 p.m.	Cut vago-sympathetics and excised stellate ganglia		
3:30 p.m.	Before stimulation of sciatic[4]	145	52
	20 seconds after beginning stimulation	169	94
3:35 p.m.	Before weaker stimulation of sciatic	152	63
	54 seconds after beginning stimulation	151	63
3:40 p.m.	Prepared peripheral end of right splanchnic in thorax		
3:42 p.m.	Before stimulation of right splanchnic	146	35
	40 seconds after beginning stimulation	192	76
	Good eye reactions in about 40 seconds		
3:45 p.m.	Before stimulation of right splanchnic; right adrenal vein clipped	157	44
	20 seconds after beginning stimulation	184	
	30 seconds after beginning stimulation	207	82
	No eye reactions		
3:50 p.m.	Before stimulation of right splanchnic; both adrenal veins clipped	150	44
	40 seconds after beginning stimulation	199	84
	No eye reactions		
3:55 p.m.	Before stimulation of right splanchnic	146	44
	30 seconds after beginning stimulation	200	68
	Good eye reactions occurred in 30–35 seconds		

In the above experiment (cat 195) the abdomen was opened, the lumbar vein tied just before it crosses the left adrenal and the left splanchnic stimulated several times before the vago-sympathetics were cut and the stellate ganglia excised. The central end of the sciatic was then stimulated and caused an acceleration of 24 beats and a rise of blood pressure from 52 to 94 mm. of mercury, showing again that Cannon's statement that the heart reaction is scarcely ever obtained with sciatic stimulation after opening the abdomen is unfounded.

[4] Eye reactions with sciatic stimulation as described in protocol of cat 190, p. 319.

Of course when a weak enough stimulus was employed (5 minutes thereafter) the heart rate remained unchanged, but so did the blood pressure. Precisely the same result was obtained with stimulation of the peripheral end of the splanchnic, a stimulus which failed to cause any appreciable rise of blood pressure (as at 3:05 p.m.) also caused little if any acceleration of the heart. Whether the corresponding adrenal vein was open or clipped seemed to have no influence on the maximum acceleration. Indeed in the first splanchnic stimulation (at 2:55 p.m.) with the vein open the maximum acceleration was only 14 beats, whereas in the next stimulation, with the vein clipped, it was 25 beats per minute. Yet the eye reactions, which were very good in the first case, were abolished in the second. The eye reactions are universally admitted to be due to epinephrin, when elicited by stimulation of the peripheral end of the splanchnic. How is it possible to believe that when they are negative, while the heart acceleration is even greater than when they were strongly positive, the acceleration of the heart is a specific reaction for epinephrin?

In another cat (196), in which the left superior cervical ganglion had been excised 20 days previously, the vago-sympathetics were cut, the stellate ganglia excised and the abdomen opened under urethane at the beginning of the experiment. The lumbar veins, just before crossing the adrenals, and the renal arteries and veins were tied on both sides and both adrenal veins prepared for clipping. The peripheral end of the left sympathetic in the thorax was prepared for stimulation and the central end of a sciatic. Stimulation of the left sympathetic with the adrenal veins open gave a good pupil reaction in 8 seconds. The heart rate, which was 212 before stimulation, reached a maximum of 255 beats per minute (counting from 16 seconds after beginning of stimulation). The blood pressure rose from 72 to 150 mm. of mercury. The left sympathetic was now stimulated with the left adrenal vein clipped. There was no pupil reaction. The heart rate increased from 201 to a maximum of 260 beats per minute (counting from 16 seconds after the beginning of stimulation) and the blood pressure from 94 to 134 mm. of mercury. Then the left sympathetic was again stimulated with both adrenal veins clipped. There was no pupil reaction but the heart rate increased from 200 to a maximum of 245 beats a minute and the blood pressure from 58 to 107 mm. of mercury. Is not this again quite inconsistent with the view that the heart acceleration constitutes a quantitative reaction by which the output of epinephrin can be determined?

The next protocol (from cat 200) illustrates the general parallelism between the acceleration of the heart elicited by sciatic stimulation and the rise of blood pressure, and the absence of any demonstrable influence of clipping of the adrenal veins on either the maximum acceleration or the increase of blood pressure.

Protocol. Cat 200; female; weight, 1.47 kgm. Left superior cervical ganglion excised 16 days previously. Under urethane anesthesia cut vago-sympathetics; excised stellate ganglia; prepared central end of sciatic for stimulation.

		Rate	*Pressure*
10:00 a.m.	Before sciatic stimulation[5]	210	84
	Counts of successive portions of the curve after beginning of stimulation (with progressive increase in strength)	211	84
		215	102
		230	112
		230	128
		244	128
10:05 a.m.	Before sciatic stimulation	214	100
	Counts of successive portions of the curve with stimulation as above	224	117
		240	126
		240	118
10:10 a.m.	Prepared adrenal veins for clipping (extraperitoneally)		
10:15 a.m.	Before sciatic stimulation	217	84
	Successive counts after beginning of stimulation as above	223	100
		234	110
		248	110
10:20 a.m.	Before sciatic stimulation; both adrenal veins clipped.	206	70
	Successive counts after beginning of stimulation as above	212	88
		222	89
		223	88
		225	86
	Just after release of adrenal veins	228	95
	15 seconds after release of adrenal veins	245	104
10:25 a.m.	Before sciatic stimulation	214	80
	Successive counts as above during stimulation	216	88
		239	104
		253	117
10:30 a.m.	Before sciatic stimulation; both adrenal veins clipped.	215	77
	During stimulation as above	227	84
		228	98
	Just after release of adrenal veins	236	96
	12 seconds after release of adrenal veins	239	100

In the last experiment (cat 193) to be quoted in this section of the paper, only the vago-sympathetics were cut at the beginning of the experiment, and the abdomen was opened.

[5] The effects on the eye were those described in the footnote to protocol of cat 190, p. 319.

Protocol. Cat 193; male; weight, 2.7 kgm. Left superior cervical ganglion excised 10 days previously. Under urethane (5 gm.) opened abdomen; prepared peripheral end of left splanchnic for stimulation; cut vago-sympathetics.

		Rate	*Pressure*
12:08 p.m.	Before stimulation of splanchnic; left adrenal vein clipped	157	98
	11 seconds after beginning stimulation	175	
	18 seconds after beginning stimulation	180	145
	No eye reactions during stimulation; after release of clip good pupil and nictitating reactions in 9.2 seconds		
12:10 p.m.	Before stimulation of splanchnic	162	96
	10 seconds after beginning stimulation	209	136
	Good pupil and nictitating reactions in 11.5 seconds		
12:15 p.m.	Before stimulation of splanchnic; left adrenal vein clipped	172	120
	17 seconds after beginning stimulation	190	162
	No eye reactions during stimulation; on release of clip good pupil and nictitating reactions in 8.2 seconds		
12:20 p.m.	Excised stellate ganglia		
12:40 p.m.	Before stimulation of splanchnic; left adrenal vein clipped	185	114
	18 seconds after beginning stimulation	201	144
	No eye reactions during stimulation; on release of clip good pupil and nictitating reactions in 11 seconds		
12:50 p.m.	Stimulated splanchnic with left adrenal vein and coeliac and superior mesenteric arteries clipped.		
	Before stimulation	203	134
	17 seconds after beginning stimulation	202	136
	No eye reactions during stimulation; on release of adrenal vein good pupil and nictitating reactions in 10.6 seconds		
	Before removal of adrenal clip	202	121
	12 seconds after release of adrenal vein	238	128
	Removed clips from coeliac and superior mesenteric		
1:00 p.m.	Prepared central end of sciatic for stimulation		
1:02 p.m.	Before stimulation of sciatic	174	78
	11 seconds after beginning stimulation	184	102
	20 seconds after beginning stimulation	194	
	34 seconds after beginning stimulation	200	104
1:20 p.m.	Before stimulation of sciatic	184	68
	16 seconds after beginning stimulation	200	108

The effects of stimulating the splanchnic, with the corresponding adrenal vein open and clipped, were not obviously different from those in the other experiments, where the stellate ganglia had been excised at the beginning, nor did removal of the stellate ganglia cause any essential change. The failure of the heart reaction with splanchnic

stimulation when the coeliac and superior mesenteric arteries were clipped is clearly associated with the absence of a rise of pressure when the splanchnic area is thus eliminated. On removal of the clip from the adrenal vein the usual good eye reactions were obtained and also an acceleration of the heart due to release of the epinephrin pent up in the adrenal vessels.

It may be tedious to point out again that an hour and a half after the abdomen had been opened, with one splanchnic cut, after all the clipping and unclipping of the adrenal veins and of the coeliac and superior mesenteric arteries (and a good many observations actually made have been omitted from the protocol to save space) stimulation of the sciatic still gave a good acceleration of the heart, although, according to Cannon, no reaction ought to have been obtained. In view of such facts what becomes of Doctor Cannon's assertion, unsupported by any evidence that when we collect adrenal vein blood from a cava pocket, the "peculiar" conditions of our experiments do not permit of the demonstration that with stimulation of sensory nerves there is a vast outpouring of epinephrin from the adrenals, a demonstration which he obtains by a misinterpretation of a heart reaction equally well elicited whether the adrenal veins are open or clipped off, whether the eye (sensitized by removal of the superior cervical ganglion) is giving negative or positive reactions for epinephrin? Since, however, Doctor Cannon maintains that the mere clipping or tying of the adrenal veins need not interfere in the least with the passage of epinephrin to the heart, and has even convinced himself that adrenalin, injected into the lumbar vein crossing the adrenal after it has been ligated on each side of the gland, passes so freely into the circulation that it causes a large rise of blood pressure, evidence of another kind will now be given that the acceleration of the heart relied upon by him to prove a markedly augmented rate of epinephrin output when the sciatic nerve is stimulated has no such significance.

EXPERIMENTS ON ANIMALS WITH ONE ADRENAL REMOVED AND THE NERVES OF THE OTHER CUT

We have previously shown (13) that after this operation the epinephrin output is either greatly reduced or abolished, within the limits of sensitiveness of the test objects (rabbit's intestine and uterus segments) employed to detect and estimate it. It was therefore of interest to see whether the acceleration of the heart would

be obtained in these animals by stimulation of sensory nerves. The result was positive in all the cats, a good acceleration being elicited by stimulation of the sciatic. For example, in cat 201, a female weighing 2.575 kgm., the right adrenal was removed, the nerves of the left including the splanchnic cut. A portion of the left semilunar ganglion was removed. The left superior cervical ganglion was excised at the same time. Eighteen days thereafter, the animal being in good condition, the vago-sympathetics were cut and the stellate ganglia removed under urethane and the central end of the left sciatic nerve prepared for stimulation. A blood pressure tracing was taken as usual from the right carotid. Stimulation of the sciatic caused the heart rate to increase from 177 before stimulation to a maximum rate of 211 beats per minute. The blood pressure increased from 54 mm. before stimu-

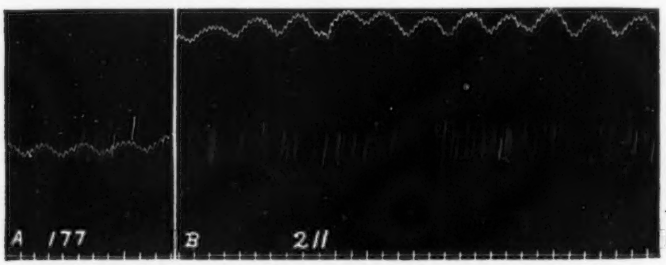

Fig. 4. Parts of blood pressure tracings from cat 201. *A*, before and *B*, a portion commencing 20 seconds after beginning of sciatic stimulation. Reduced to three-fifths.

lation to a maximum of 132 mm. of mercury. The acceleration and the blood pressure increased together. Successive portions of the curve starting from the point of stimulation yielded the following heart rates and blood pressures 180 (72,) 193 (97), 202 (107), 209 (118), 209 (132). The blood pressures are in parentheses. A sample of the curve is reproduced in figure 4. The effect of sciatic stimulation on the pupils did not differ materially from that already described in cats in which the epinephrin output had not been interfered with (see footnote to protocol of cat 190). The nictitating membrane did not move. The abdomen was afterwards opened, a short cava pocket made and adrenal vein blood collected, which was assayed on rabbit segments. It was shown that the epinephrin output could not have been more than one-fiftieth of the average output per kilogram per minute, under

the conditions of our experiments. The preliminary operation had, therefore, effectively severed the epinephrin-secretory nerves of the left adrenal. In spite of this and also in spite of the fact that one splanchnic had been divided, and with both splanchnics intact a greater reflex rise of blood pressure might have been obtained, a maximum acceleration of the heart of 32 to 34 beats was elicited by stimulation of the sciatic. According to Cannon, the whole of the acceleration must have been due to a great outpouring of epinephrin from the left adrenal, reflexly stimulated along efferent nerve paths which had been divided 18 days before, and which certainly had not regenerated in that time.

In another cat (202), a female weighing 2.545 kgm., the right adrenal was removed and the nerves of the left cut. The left superior cervical ganglion was excised at the same time. Eighteen days thereafter the heart was denervated under urethane and blood pressure tracings taken from the right carotid. Stimulation of the central end of the sciatic caused an acceleration of the heart rate from 145 per minute before stimulation to 161. The blood pressure rose from 92 to 134 mm. of mercury. Successive portions of the curve, from the beginning of stimulation, yielded the following heart rates and blood pressures: 152 (100), 155 (110), 157 (130), 160 (126), 161 (134). The pupil reactions were as previously described in the footnote to the protocol of cat 190. There was no movement of the nictitating membrane. After the sciatic stimulations the abdomen was opened, and 3 specimens of adrenal vein blood collected—the first, about 1 gram (discarded), the second, 2.9 grams in 4 minutes (0.75 gm. per minute), the third 5.1 grams in 9 minutes (0.57 gm. per minute). No evidence was obtained with rabbit segments that the blood contained any epinephrin. It was shown that the third specimen could not have contained 1:70,000,000 epinephrin, i.e., the output could not have been 0.000007 mgm. per minute for the cat, or 0.0000027 mgm. per kilogram per minute. In other words it could not have been one-hundredth of the average normal output, and there was no evidence that any epinephrin was being given off. The interval after the preliminary operation was quite short in this animal, 8 days, which possibly may be an unfavorable condition for obtaining a large heart reaction, although we have no evidence as to this.

In the next experiment (cat 215) the animal was allowed to survive for 2 months.

Protocol. Cat 215; female; weight 2.38 kgm.

May 17, 1918. Right adrenal excised, left denervated and right superior cervical ganglion excised.

July 15, 1918. Under urethane (5 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
11:15 a.m.	Before sciatic stimulation for 38 seconds (8 cm.)[6]	219	134
	During first 14 seconds of stimulation	232	162
	During next 13 seconds of stimulation	238	170
	During next 13 seconds	244	172
	During next 20 seconds	228	147
11:20 a.m.	Before sciatic stimulation for 33 seconds (6 cm.)	222	144
	During first 14 seconds of stimulation	240	180
	During next 14 seconds of stimulation	248	196
	During next 15 seconds	249	180
11:25 a.m.	Before sciatic stimulation for 26 seconds (6.5 cm.)	219	152
	During first 8 seconds of stimulation	226	178
	During next 7 seconds of stimulation	230	182
	During next 12 seconds	246	190
11:30 a.m.	Before sciatic stimulation for 40 seconds (6 cm.)	224	148
	During first 13 seconds of stimulation	239	177
	During next 14 seconds of stimulation	252	180
	During next 13 seconds of stimulation	251	182
	During next 10 seconds	248	168
11:35 a.m.	Before sciatic stimulation for 37 seconds (4 cm.)	222	148
	During first 12 seconds of stimulation	239	173
	During next 11 seconds of stimulation	244	180
	During next 11 seconds of stimulation	252	180
	During next 14 seconds	249	168

Later on the abdomen was opened and adrenal vein blood collected. Assays of the adrenal blood released from a cava pocket were also made by the pupil reaction.

The protocol shows that stimulation of the sciatic caused good acceleration of the heart with corresponding changes in the blood pressure. The rabbit intestine assay of the adrenal blood demonstrated that a substantial output of epinephrin was still going on. The concentration of the second specimen was about 1:6,500,000 and that of the third specimen about 1:3,750,000, corresponding in either case to an output of 0.00008 mgm. per minute for the cat, or 0.00003 mgm. per kilogram per minute, one-seventh or one-eighth of the average normal output. It is impossible to say whether an unusually large proportion of the secretory fibers of the left adrenal had escaped

[6] Eye reactions, as described in footnote to protocol of cat 190, p. 319. The distance between the coils is given in brackets.

section at the preliminary operation, or whether some regeneration had occurred. In any case the innervation of the one adrenal remaining must still have been seriously crippled. Yet the heart reaction on which Cannon relies as an "indicator of adrenal secretion" was as well obtained with sciatic stimulation as in normal animals, corresponding with the general excellent condition of the cat two months after the primary operation.

In two cats operated on in the same way but only a relatively short time before (8 days and 7 days), the experiment was completed by excising the remaining (already denervated) adrenal. It was not thought advisable in these cats to complicate the experiment by attempting to estimate the residual epinephrin output, if any, on the adrenal vein blood. But it was shown that the epinephrin store of the adrenal constituted a full load, a good indication that protection of the gland from depletion during the experiment was relatively complete.

Protocol. Cat 444; male; weight, 2.9 kgm. Right adrenal excised and left denervated 8 days, left superior cervical ganglion excised 14 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	*Pressure*
10:40 a.m.	Before sciatic stimulation (8 cm.)	133	132
	2 seconds after beginning stimulation	130	128
	30 seconds after beginning stimulation	132	130
10:45 a.m.	Before sciatic stimulation (5 cm.)	133	112
	6 seconds after beginning stimulation	140	
	40 seconds after beginning stimulation	152	92
	60 seconds after beginning stimulation	145	106
10:50 a.m.	Before sciatic stimulation (8 cm.)	127	108
	3 seconds after beginning stimulation	132	
	30 seconds after beginning stimulation	136	100
	45 seconds after beginning stimulation	131	104
10:55 a.m.	Before sciatic stimulation (6 cm.)	121	120
	5 seconds after beginning stimulation	130	126
11:10 a.m.	Excised left adrenal (extraperitoneally)		
11:15 a.m.	Before sciatic stimulation (8 cm.)	130	103
	3 seconds after beginning stimulation	133	96
	23 seconds after beginning stimulation	140	
	60 seconds after beginning stimulation	144	114
11:48 a.m.	Opened abdomen, tied coeliac axis and superior mesenteric artery		
11:50 a.m.	Before sciatic stimulation (6 cm.)	139	74
	8 seconds after beginning stimulation	144	74
	3 seconds after stopping stimulation	142	72
	40 seconds after stopping stimulation	134	70

11:58 a.m.	Before sciatic stimulation (4 cm.)	123	72
	2 seconds after beginning stimulation	130	77
	15 seconds after beginning stimulation	133	80
	40 seconds after beginning stimulation	137	81
	60 seconds after beginning stimulation	138	78
12:05 p.m.	Before sciatic stimulation (8 cm.)	130	76
	2 seconds after beginning stimulation	133	80
	30 seconds after beginning stimulation	135	82

In cat 444 the greatest acceleration caused by sciatic stimulation before excision of the remaining adrenal was 19 beats. With repeated stimulations the acceleration effect declined. The last stimulation before excision gave only 9 beats. A weaker stimulation just after excision gave a maximum acceleration of 14 beats, and another stronger stimulation gave a maximum acceleration of 15 beats. Even after the abdomen was opened and the superior mesenteric artery and coeliac axis tied, a maximum acceleration of 15 beats was obtained in the absence of the adrenals.

In cat 441, also, while the greatest acceleration before excision of the remaining adrenal (17 beats) was not reached afterwards, the acceleration immediately after excision was the same as that obtained just before it (9 beats), although the stimulation in the latter case was the stronger.

Protocol. Cat 441; female; weight, 1.66 kgm. Right adrenal excised and left denervated 7 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	*Pressure*
10:55 a.m.	Before sciatic stimulation (10 cm.)	219	100
	6 seconds after beginning stimulation	229	116
	20 seconds after beginning stimulation	228	
10:58 a.m.	Before sciatic stimulation (8 cm.)	217	115
	6 seconds after beginning stimulation	223	122
11:02 a.m.	Before sciatic stimulation (6 cm.)	208	106
	10 seconds after beginning stimulation	215	118
	30 seconds after beginning stimulation	213	
11:06 a.m.	Before sciatic stimulation (6 cm.)	211	109
	5 seconds after beginning stimulation	217	
	18 seconds after beginning stimulation	228	120
	28 seconds after beginning stimulation	227	
11:10 a.m.	Before sciatic stimulation (10 cm.)	207	113
	5 seconds after beginning stimulation	215	116
11:15 a.m.	Before sciatic stimulation (8 cm.)	206	109
	5 seconds after beginning stimulation	222	139

		Rate	*Pressure*
11:20 a.m.	Before sciatic stimulation (7 cm.)	216	116
	5 seconds after beginning stimulation	224	148
11:25 a.m.	Before sciatic stimulation (6 cm.)	212	98
	5 seconds after beginning stimulation	221	119
11:40 a.m.	Excised left adrenal (lumbar route)		
11:42 a.m.	Before sciatic stimulation (8 cm.)	214	86
	5 seconds after beginning stimulation	220	
	20 seconds after beginning stimulation	223	114
11:45 a.m.	Before sciatic stimulation (10 cm.)	211	84
	5 seconds after beginning stimulation	214	100
	23 seconds after beginning stimulation	215	
11:51 a.m.	Before sciatic stimulation (9 cm.)	210	70
	6 seconds after beginning stimulation	214	88
11:55 a.m.	Before sciatic stimulation (7 cm.)	208	70
	6 seconds after beginning stimulation	210	84
11:58 a.m.	Before sciatic stimulation (8 cm.)	211	68
	16 seconds after beginning stimulation	218	97
12:00 m.	Prepared central end of left sciatic for stimulation		
12:05 p.m.	Before stimulation of left sciatic (9 cm.)	214	68
	30 seconds after beginning stimulation	217	72
12:10 p.m.	Before stimulation of right sciatic (10 cm.)	215	68
	5 seconds after beginning stimulation	216	76
12:15 p.m.	Before stimulation of right sciatic (8 cm.)	236	55
	5 seconds after beginning stimulation	242	62
	11 seconds after beginning stimulation	245	

The left adrenal weighed 0.226 gm. and contained 0.23 mgm. of epinephrin at end of experiment.

In the last cat (450) of this series to be mentioned a longer period (34 days) was allowed to elapse between the primary operation and the experiment, in order that the animal might have more fully recovered.

Protocol. Cat 450; male; weight, 2.96 kgm. Right adrenal excised and left denervated (with section of left splanchnic as usual) 34 days previously. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
11:10 a.m.	Before sciatic stimulation (10 cm.)	173	102
	During first 18 seconds of stimulation	178	114
	During next 20 seconds of stimulation	182	114
	Just after end of stimulation	185	
	30 seconds after end of stimulation	180	
11:14 a.m.	Before sciatic stimulation (9 cm.)	174	100
	During first 12 seconds of stimulation	178	
	During next 16 seconds of stimulation	183	133
	Just after end of stimulation	191	126
	20 seconds after end of stimulation	189	129

		Rate	*Pressure*
11:18 a.m.	Before sciatic stimulation (8 cm.)	171	97
	During first 16 seconds of stimulation	178	
	During next 17 seconds of stimulation	187	134
	Just after end of stimulation	192	134
	20 seconds after end of stimulation	190	
11:40 a.m.	Excised left adrenal (extraperitoneally)		
11:43 a.m.	Before sciatic stimulation (9 cm.)	174	94
	During first 15 seconds of stimulation	179	130
	During next 20 seconds of stimulation	189	127
11:48 a.m.	Before sciatic stimulation (8 cm.)	174	103
	During first 14 seconds of stimulation	178	132
	During next 20 seconds of stimulation	191	144
	Just after end of stimulation	200	150
	15 seconds after end of stimulation	198	128
	35 seconds after end of stimulation	194	127
11:58 a.m.	Before sciatic stimulation (7 cm.)	177	96
	During first 17 seconds of stimulation	181	128
	During next 20 seconds of stimulation	192	138
	Just after end of stimulation	200	122
	20 seconds after end of stimulation	195	120
12:10 p.m.	Prepared peripheral end of right splanchnic (in thorax)		
12:12 p.m.	Before splanchnic stimulation (10 cm.)	167	70
	During first 20 seconds of stimulation	176	96
	During next 20 seconds of stimulation	184	104
	Just after end of stimulation	186	92
12:15 p.m.	Before splanchnic stimulation (8 cm.)	177	61
	During first 20 seconds of stimulation	175	70
	During next 20 seconds of stimulation	185	90
	Just after end of stimulation	189	86
12:21 p.m.	Before splanchnic stimulation (7 cm.)	178	60
	During first 16 seconds of stimulation	179	93
	During next 20 seconds of stimulation	186	108
	Just after end of stimulation	187	96
12:26 p.m.	Before sciatic stimulation (7 cm.)	179	62
	During first 20 seconds of stimulation	182	83
	During next 20 seconds of stimulation	180	72
12:35 p.m.	Cut left splanchnic in thorax		
12:36 p.m.	Before sciatic stimulation (7 cm.)	181	63
	During first 18 seconds of stimulation	182	82
	During next 20 seconds of stimulation	181	78
	Just after end of stimulation	184	74
1:01 p.m.	Before splanchnic stimulation (7 cm.)	183	46
	During first 16 seconds of stimulation	184	78
	During next 20 seconds of stimulation	196	88
	Just after end of stimulation	208	74
	15 seconds after end of stimulation	209	65
	22 seconds after end of stimulation	212	60

It will be seen from the protocol that a good acceleration was obtained on sciatic stimulation before removal of the remaining (already denervated) adrenal, (12 beats, 17 beats, 21 beats per minute in successive observations with different strength of stimulation). After removal of the adrenal the accelerations obtained were fully as great as before (15 beats, 26 beats, 23 beats per minute). When, however, in the absence of the adrenals, the right splanchnic was cut in the thorax sciatic stimulation caused practically no acceleration, and the rise of pressure was, of course, much reduced (fig. 5). This was not due to any change in the condition of the animal which rendered direct stimulation of the splanchnic less effective, for the rise of pressure and the acceleration on stimulating the peripheral end of the right splanchnic

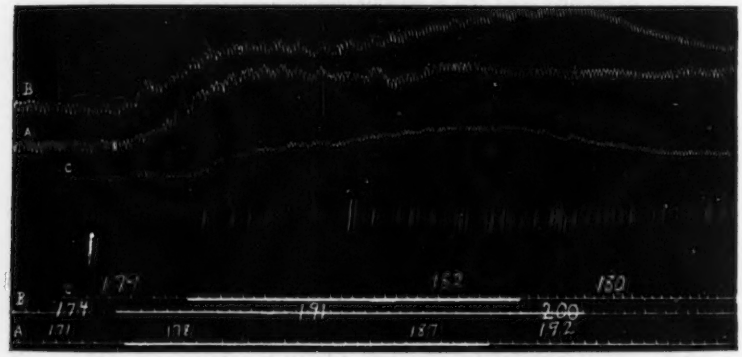

Fig. 5. Blood pressure curves from cat 450. *A*, sciatic stimulation before and *B*, after excision of remaining (already denervated) adrenal; *C*, after section of remaining splanchnic. Reduced to three-fifths.

was quite as great as before the last sciatic stimulation (as much as 29 beats per minute), (fig. 6). The failure of the heart reaction with sciatic stimulation after section of the right splanchnic (the left had been divided at the primary operation) is precisely what Cannon describes as occurring after removal of the adrenals. But in this animal the removal of the remaining adrenal did not affect the heart reaction, while subsequent section of the remaining splanchnic abolished it. This is incompatible with Cannon's interpretation of the failure of the reaction after section of the splanchnics as due entirely to interference with the epinephrin output. In the present experiment, to be logical, he would have to attribute the result to the loss of something coming from the liver (sugar?) or from the intestines, mobilized reflexly through the splanchnic.

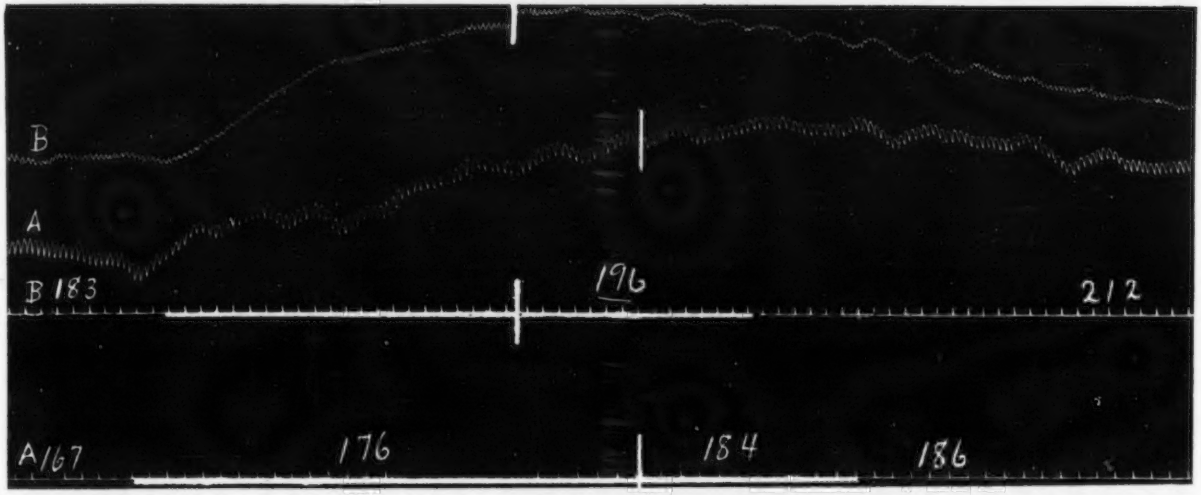

Fig. 6. Blood pressure curves from cat 450. *A*, splanchnic stimulation after excision of remaining (already denervated) adrenal; *B*, same, with stronger stimulus, 50 minutes later (7 seconds of each trace cut out to save space).

EXPERIMENTS WITH AN INTERVAL BETWEEN REMOVAL OF THE TWO ADRENALS

To avoid division of one splanchnic and to make the first operation less severe than the ordinary operation for suppression of the epinephrin output, while making the second operation less severe than the removal of both adrenals at one time, a number of experiments were performed in which one adrenal was removed, and then, after an interval of 3 to 13 days, the observations on sciatic stimulation with denervation of the heart and excision of the remaining adrenal were made. In cat 440, for example, the experiment was performed 6 days after the removal of the right adrenal.

Protocol. Cat 440; male; weight, 2.31 kgm. Right adrenal excised 6 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	*Pressure*
10:45 a.m.	Before sciatic stimulation (10 cm.)	226	134
	5 seconds after beginning stimulation	256	168
10:49 a.m.	Before sciatic stimulation (8 cm.)	220	128
	6 seconds after beginning stimulation	253	171
	15 seconds after end of stimulation	264	156
11:00 a.m.	Excised left adrenal (extraperitoneally)		
11:02 a.m.	Before sciatic stimulation (8 cm.)	212	124
	5 seconds after beginning stimulation	242	
	15 seconds after beginning stimulation	255	156
	23 seconds after beginning stimulation	250	156
	37 seconds after beginning stimulation	228	138
	60 seconds after beginning stimulation	218	134
11:05 a.m.	Before sciatic stimulation (6 cm.)	213	119
	6 seconds after beginning stimulation	252	154
	28 seconds after beginning stimulation	247	
11:09 a.m.	Before sciatic stimulation (4 cm.)	212	118
	5 seconds after beginning stimulation	230	133
	22 seconds after beginning stimulation	248	153
11:20 a.m.	Before sciatic stimulation (8 cm.)	221	112
	6 seconds after beginning stimulation	236	130
	22 seconds after beginning stimulation	247	140
11:25 a.m.	Before sciatic stimulation (6 cm.)	230	118
	8 seconds after beginning stimulation	241	130
11:28 a.m.	Before sciatic stimulation (4 cm.)	225	133
	7 seconds after beginning stimulation	231	146
	43 seconds after beginning stimulation	227	130
11:45 a.m.	Exposed cord in midcervical region		
11:50 a.m.		208	90

		Rate	*Pressure*
11:55 a.m.	Before total asyphxia (for 45 seconds)	207	84
	3 seconds after beginning asphyxia	204	
	10 seconds after beginning asphyxia	225	88
	55 seconds after beginning asphyxia	210	88
12:05 a.m.	3 minutes after transection of cord between 4th and 5th cervical segments	193	51

As will be seen from the protocol, excellent heart reactions were obtained both before and after the removal of the remaining adrenal in cat 440. It is to be particularly noted that excision of the left adrenal left the blood pressure and heart rate practically unchanged.

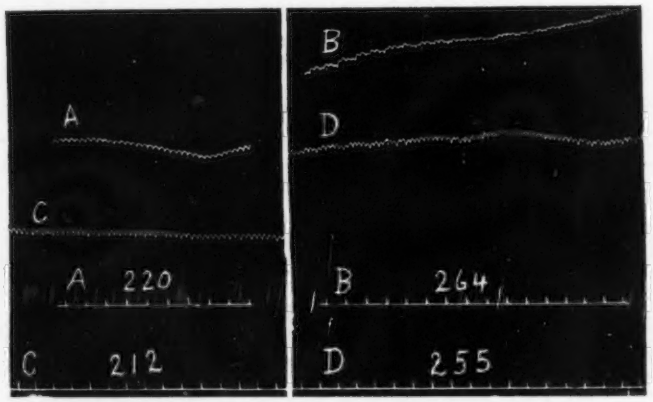

Fig. 7. Parts of blood pressure tracings from cat 440. *A*, before and *B*, a portion commencing 15 seconds after beginning of sciatic stimulation, before excision of remaining adrenal; *C*, before and *D*, 15 seconds after beginning of sciatic stimulation, after excision of remaining adrenal. Zero line moved up 41 mm.

The maximum acceleration for the last sciatic stimulation prior to removal of the left adrenal was 33 beats per minute, and for the first sciatic stimulation after removal of the adrenal 42 beats, the maximum pulse rate reached being practically identical in the two cases (253, 250). Samples of the curves used in counting the pulse rate are given in figure 7. The greater portion of each of the two curves, much reduced, is reproduced in figure 8, to show that excision of the second adrenal has not in any way essentially changed the vascular reaction. Here was a cat, then, without adrenals in which the acceleration produced by stimulation of the sciatic was actually greater than that

produced by a similar stimulation while one adrenal was still intact. Yet, according to Cannon, the last acceleration, like the first, must have been due solely to augmented epinephrin output from the adrenals. It should be noted that the exposure of the cord in the midcervical region led to a drop of blood pressure to 90 mm. of mercury and a corresponding slowing of the heart to 208 beats a minute. Subsequent transection of the cord caused the blood pressure to fall to 51 mm. of mercury and the heart rate to 193. It must be remembered that the adrenals were out, and the decrease in the heart rate can have nothing to do with absence of adrenal epinephrin. Cannon has emphasized the fact that after removal of the adrenals the pulse rate drops. Our observations show that this is always true provided the blood pressure

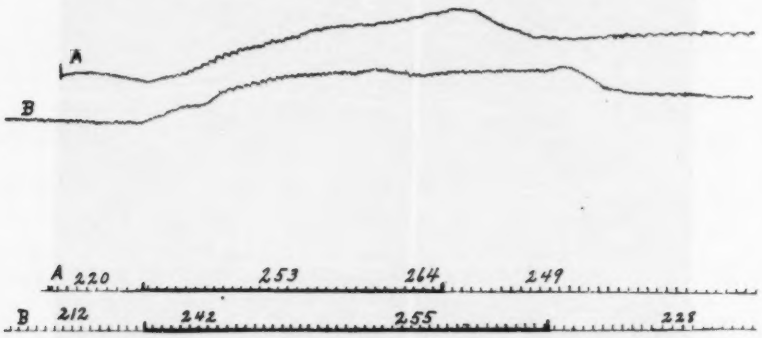

Fig. 8. Blood pressure curves from cat 440. *A*, sciatic stimulation before and *B*, after removal of remaining adrenal. Reduced to one-half.

falls decidedly. But if the pressure is maintained there is not necessarily any sensible slowing of the heart. The diminution in the pulse rate is, therefore, no index of the previous rate of output of epinephrin. This statement of observed facts is made without prejudice to the question whether the epinephrin liberated at the ordinary rate, under our experimental conditions, is capable of exerting an influence upon the heart, including its rate. We have brought forward some evidence that there is such an influence. But it may not be obvious after suppression of the epinephrin output when the blood pressure is well maintained. In cat 440 repeated stimulation of the sciatic caused some exhaustion of the heart reaction after removal of the last adrenal. But this, of course, is true of all reflexes, and in the case of the heart reaction can occur also with intact adrenals.

In the next experiment (cat 443) an example is given in which the heart acceleration elicited by sciatic stimulation was small from the beginning, but quite as good reactions were obtained after removal of the second adrenal as before (greatest acceleration before removal of the adrenal, 7 beats in one observation and 9 beats in another; after removal, 10 beats in one observation and 11 beats in another). It will be noted that as the blood pressure continued to fall progressively the heart rate diminished also, and this was not related to the adrenalectomy. Thus half an hour after removal of the adrenal, the pulse rate was 160 and the blood pressure 76. When the blood pressure had fallen to 50 the heart rate was 147. Before the removal of the adrenal the pulse rate was 180, 179 and 200 in 3 observations. After the adrenalectomy it was 182, the blood pressure being 83 mm. of mercury instead of 110 mm. at the last observation. As in other protocols counts of the heart rate at different parts of the curve and the results of stimulation of different strengths are given in order to show that the maximum accelerations quoted were really the maximum obtainable, under our conditions, in each animal.

Protocol. Cat 443; female; weight, 1.53 kgm. Right adrenal excised 7 days, left superior cervical ganglion excised 13 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
10:55 a.m.	Before sciatic stimulation (10 cm.)[7]	180	116
	6 seconds after beginning stimulation	187	148
10:59 a.m.	Before sciatic stimulation (9 cm.)	179	106
	6 seconds after beginning stimulation	183	128
11:04 a.m.	Before sciatic stimulation (9 cm.)	200	110
	20 seconds after beginning stimulation	209	122
	45 seconds after beginning stimulation	205	116
11:15 a.m.	Excised left adrenal (extraperitoneally)		
11:16 a.m.	Before sciatic stimulation (10 cm.)	182	83
	5 seconds after beginning stimulation	180	85
11:20 a.m.	Prepared central end of right sciatic		
11:27 a.m.	Before stimulation of left sciatic (8 cm.)	165	80
	23 seconds after beginning stimulation	168	100
11:43 a.m.	Before stimulation of right sciatic (6 cm.)	161	75
	2 seconds after beginning stimulation	160	
	6 seconds after beginning stimulation	164	
	17 seconds after beginning stimulation	170	94
	3 seconds after end of stimulation	171	

[7] The eye reactions were the same as described in footnote to protocol of cat 190, p. 319.

		Rate	Pressure
11:46 a.m.	Before sciatic stimulation (6 cm.)	160	76
	10 seconds after beginning stimulation	169	98
	6 seconds after end of stimulation	170	
11:50 a.m.	Before stimulation of left sciatic (6 cm.)	155	62
	6 seconds after beginning stimulation	157	70
12:07 p.m.	Before stimulation of left sciatic (6 cm.)	147	50
	7 seconds after beginning stimulation	151	
	30 seconds after beginning stimulation	153	56

In both of the experiments hitherto cited in this section (cats 440 and 443) the blood pressure after removal of the second adrenal remained good, practically unchanged in the first cat and only moderately lowered in the other. In the next experiment (cat 438) a considerable fall of pressure accompanied the operation for removal of the second adrenal. There is no reason to attribute this to the fact that the operation was done by the abdominal route. A similar fall of pressure occurred in cat 439 after removal of the second adrenal extraperitoneally by the lumbar route.

Protocol. Cat 438; female, weight 1.91 kgm. Right adrenal excised 3 days previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
12:08 p.m.	Before sciatic stimulation (6 cm. to 4 cm.)	189	122
	After increasing strength of stimulus (5 cm.)	194	
	After increasing strength of stimulus (4 cm.)	200	
	At end of stimulation	213	154
12:30 p.m.	Before sciatic stimulation (6–4 cm.)	206	110
	4 seconds after beginning stimulation	220	140
	After increasing strength of stimulus (4 cm.)	237	178
12:45 p.m.	Opened abdomen, excised left adrenal		
12:50 p.m.	Before sciatic stimulation (4 cm.)	130	40
	During first 15 seconds stimulation	138	
	After increasing strength of stimulus	143	52
	After further increasing strength of stimulus	150	64
1:03 p.m.	Before sciatic stimulation (6–4 cm.)	161	
	5 seconds after beginning stimulation	170	
1:10 p.m.	Before sciatic stimulation (6–4 cm.)	161	66
	5 seconds after beginning stimulation	162	
	30 seconds after beginning stimulation	166	83
1:15 p.m.	Before sciatic stimulation (4 cm.)	171	60
	10 seconds after beginning stimulation	170	74
1:20 p.m.	Before total asphyxia for 1 minute	175	64
	Counting from end of asphyxia	225	64

In cat 438 it should be noted that with the fall of pressure from well over 100 mm. to 40 mm. of mercury, associated with the adrenalectomy, the pulse rate diminished from 206 before the previous sciatic stimulation to 130. To attribute this diminution of 76 beats per minute entirely to the lack of the epinephrin from the second adrenal is, we believe, unwarranted. As the pressure gradually rose later on in the experiment, be it remembered in the absence of the adrenals, the pulse rate increased also to 161, 171 and 175 with pressures of 60 to 66. In spite of the rather low blood pressure which, however, showed a tendency to improve, a fair acceleration of the heart was elicited by stimulation of the sciatic after removal of the second adrenal (as much as 20 beats per minute, compared with 24 beats and 31 beats in two observations before the adrenalectomy).

Protocol. Cat 439; male; weight, 1.93 kgm. Right adrenal excised 5 days previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia and prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
11:30 a.m.	Before sciatic stimulation (10 cm.)	210	125
	4 seconds after beginning stimulation	217	146
	After increasing strength of stimulus (8 cm.)	234	160
11:40 a.m.	Before sciatic stimulation (6 cm.)	200	100
	3 seconds after beginning stimulation	222	142
11:50 a.m.	Excised left adrenal (extraperitoneally)		
11:53 a.m.	Before sciatic stimulation (6 cm.)	178	54
	15 seconds after beginning stimulation	181	68
	After increasing strength of stimulus (5 cm.)	180	66
12:00 m.	Before sciatic stimulation (6 cm.)	179	56
	6 seconds after beginning stimulation	183	72
12:10 p.m.	Prepared central end of right sciatic		
12:12 p.m.	Before stimulation of right sciatic (7 cm.)	180	40
	5 seconds after beginning stimulation	183	58
	After increasing strength of stimulus (3 cm.)	183	49
12:20 p.m.	Before sciatic stimulation (6 cm.)	177	40
	5 seconds after beginning stimulation	182	44
	After end of stimulation	182	40
12:25 p.m.	Before intravenous injection of Ringer	173	20
	Immediately after injection of 100 cc. Ringer	188	46
	30 seconds after end of injection	190	68

In cat 439 although the blood pressure, immediately after removal of the second adrenal, was a little higher than in cat 438, it tended to grow progressively worse, sinking at last to 20 mm. of mercury, whereas the opposite tendency was seen in cat 438. Only very trifling accelerations of the heart were caused by sciatic stimulation after the adre-

nalectomy, although before fair reactions were obtained (as much as 24 beats in one observation and 22 beats per minute in another). The changes of blood pressure produced by stimulation of the sciatic were also small. Since the heart reaction depends upon a reflex or reflexes, as already pointed out, it necessarily fails or is diminished when the reflex arcs have deteriorated under the influence of a low blood pressure and a poor blood flow. Toward the end of the experiment an injection of Ringer's solution raised the blood pressure from 20 to 68 mm. and the pulse rate from 173 to 190. Epinephrin, of course, could have nothing to do with this acceleration. That the relative failure of the heart reaction on sciatic stimulation after removal of the second adrenal was not dependent upon the impossiblity of a reflex increase in the epinephrin output but upon other, probably circulatory conditions, is indicated in the next experiment (cat 446), the last to be cited in this section.

Protocol. Cat 446; female; weight, 1.67 kgm. Right adrenal excised 6 days previously. Under urethane cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	*Pressure*
11:00 a.m.	Before sciatic stimulation (8 cm.)	185	115
	10 seconds after beginning stimulation	200	152
	20 seconds after beginning stimulation	203	
	30 seconds after beginning stimulation	204	123
11:02 a.m.	Before sciatic stimulation (9 cm.)	185	104
	10 seconds after beginning stimulation	200	130
	32 seconds after beginning stimulation	199	113
11:20 a.m.	Excised left adrenal (extraperitoneally)		
11:22 a.m.	Before sciatic stimulation (9 cm.)	148	57
	10 seconds after beginning stimulation	150	64
	34 seconds after beginning stimulation	150	61
11:30 a.m.	Intravenous injection of 100 cc. Ringer		
11:50 a.m.	Before sciatic stimulation (7 cm.)	164	80
	10 seconds after beginning stimulation	174	
	25 seconds after beginning stimulation	178	115
	40 seconds after beginning stimulation	173	
11:55 a.m.	Before sciatic stimulation (8 cm.)	161	76
	4 seconds after beginning stimulation	163	
	22 seconds after beginning stimulation	168	97
	40 seconds after beginning stimulation	162	82
12:03 p.m.	Before sciatic stimulation (6 cm.)	162	72
	5 seconds after beginning stimulation	170	88
	23 seconds after beginning stimulation	171	
	42 seconds after beginning stimulation	169	70
12:06 p.m.	Opened abdomen, tied renal arteries and veins		

		Rate	Pressure
12:11 p.m.	Clipped abdominal aorta and stimulated sciatic;		
	before stimulation (7 cm.)	171	74
	10 seconds after beginning stimulation	173	
	35 seconds after beginning stimulation	176	83
	After removal of clip	172	53
12:20 p.m.	Intravenous injection of 50 cc. Ringer		
12:25 p.m.	Clipped abdominal aorta and stimulated sciatic;		
	before stimulation (7 cm.)	170	90
	10 seconds after beginning stimulation	179	
	28 seconds after beginning stimulation	176	96
	After release of aorta	174	81
12:30 p.m.	Prepared central end of left sciatic for stimulation		
12:32 p.m.	Clipped abdominal aorta and stimulated left sciatic;		
	before stimulation (7 cm.)	173	88
	10 seconds after beginning stimulation	180	
	30 seconds after beginning stimulation	185	110
	45 seconds after beginning stimulation	180	88
	10 seconds after release of aorta	174	63
12:35 p.m.	Clipped abdominal aorta and stimulated left sciatic;		
	before stimulation (6 cm.)	163	70
	10 seconds after beginning stimulation	169	78
	31 seconds after beginning stimulation	171	60
	10 seconds after release of aorta	166	45

After removal of the second adrenal the blood pressure fell from over 100 mm. of mercury to 57 mm. and the pulse rate dropped from 185 to 147 beats per minute. Sciatic stimulation caused practically no acceleration of the heart (3 beats per minute as compared with 15 beats before the adrenalectomy) and very little rise of blood pressure (7 mm.). The pressure went on falling and Ringer's solution was injected, which brought the blood pressure up to 80 mm. Stimulation of the sciatic nerve now caused an acceleration of 14 beats per minute and a rise of blood pressure of 35 mm. of mercury. Another stimulation caused an acceleration of 12 beats when the blood pressure had been raised to 88 mm. of mercury by temporary clipping of the abdominal aorta, the clip being put on and the pressure allowed to become constant, which only required a fraction of a minute, before the beginning of stimulation. Samples of portions of the tracings used for counting the pulse rate are given in figures 9 and 10, and the whole curves (much reduced) from which these portions were taken, in figure 11.

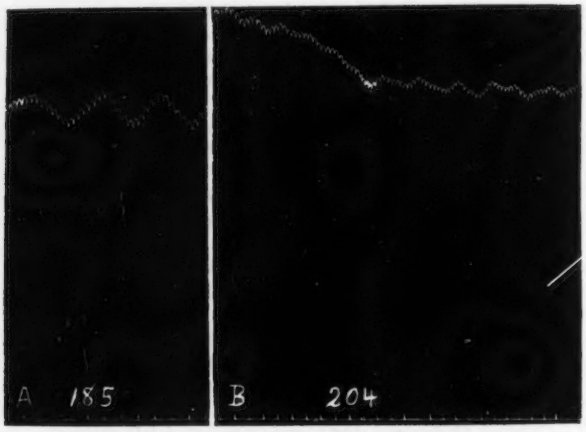

Fig. 9. Parts of blood pressure tracings from cat 446. *A*, before and *B*, a portion commencing 19 seconds after beginning of sciatic stimulation. Reduced to four-fifths.

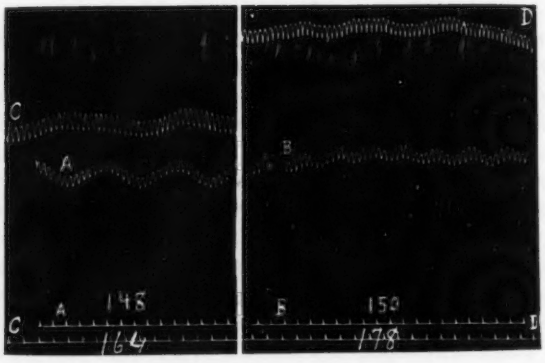

Fig. 10. Parts of blood pressure tracings from cat 446. *A*, before and *B*, a portion commencing 16 seconds after beginning of sciatic stimulation, after excision of remaining adrenal; *C*, before and *D*, 23 seconds after beginning of sciatic stimulation, after intravenous injection of Ringer. Reduced to four-fifths.

EXPERIMENTS IN WHICH BOTH ADRENALS WERE REMOVED AT ONE TIME

It seems probable that in most of the cats with one adrenal removed some time before the experiment the interval was too short for the full advantage of this procedure to be obtained, if there is an advantage. The results were, nevertheless, decisive as regards the question at issue. To check the matter a series of observations was made in which both adrenals were removed, as carefully as possible, at the time of the experiment on the denervated heart reaction. The protocol of cat 449 is cited as an example of experiments in which the adrenals were extirpated after opening the abdomen.

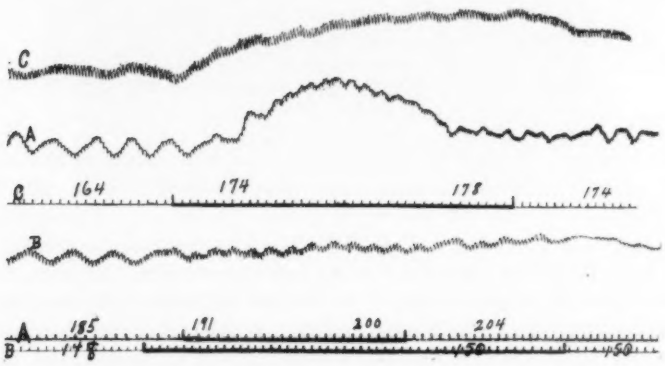

Fig. 11. Blood pressure curves from cat 446. *A*, sciatic stimulation before and *B*, after excision of remaining adrenal; *C*, after intravenous injection of Ringer. Reduced to one-half.

Protocol. Cat 449; male; weight, 3.38 kgm. Under urethane (6 gm.) cut vagosympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
12:00 m.	Before sciatic stimulation (8 cm.)	285	125
	10 seconds after beginning stimulation	293	170
	25 seconds after beginning stimulation	294	
12:12 p.m.	Before sciatic stimulation (6 cm.)	256	110
	15 seconds after beginning stimulation	280	147
	40 seconds after beginning stimulation	283	130
12:35 p.m.	Opened abdomen, excised both adrenals		
12:36 p.m.	Before sciatic stimulation (6 cm.)	228	70
	12 seconds after beginning stimulation	238	124
	35 seconds after beginning stimulation	238	90

		Rate	*Pressure*
12:50 p.m.	Before sciatic stimulation (5 cm.)	223	74
	15 seconds after beginning stimulation	241	127
	30 seconds after beginning stimulation	238	89
1:05 p.m.	Before sciatic stimulation (7 cm.)	222	73
	10 seconds after beginning stimulation	237	130
	30 seconds after beginning stimulation	234	92
1:15 p.m.	Prepared peripheral end of left splanchnic in thorax for stimulation.		
1:20 p.m.	Before splanchnic stimulation (9 cm.)	226	58
	10 seconds after beginning stimulation	231	111
	30 seconds after beginning stimulation	223	69
1:25 p.m.	Before splanchnic stimulation (7 cm.)	221	59
	8 seconds after beginning stimulation	229	110
1:40 p.m.	Before sciatic stimulation (6 cm.)	221	52
	10 seconds after beginning stimulation	231	92
	26 seconds after beginning stimulation	230	
1:45 p.m.	Before sciatic stimulation (6 cm.)	223	52
	12 seconds after beginning stimulation	232	98
	40 seconds after beginning stimulation	233	64

Two sciatic stimulations were made before extirpation of the adrenals. The first yielded a maximum acceleration of 9 beats only, but the initial heart rate was unusually great. In the second stimulation the maximum acceleration was 24 to 27 beats per minute, the initial rate being decidedly lower than in the first observation. After removal of the adrenals, accelerations of 10, 18 and 15 beats were obtained in 3 successive sciatic stimulations, and even at the end of the experiment, when the blood pressure had fallen considerably, an acceleration of 10 beats was gotten. It is scarcely necessary to point out that it would be futile to try to determine from such figures whether epinephrin was taking any sensible share in the reaction before the adrenalectomy, and if so, how much. For the maximum acceleration of which a given heart is capable at different stages in an experiment, under the influence of the changes produced by stimulation of the sciatic other than any possible change in the epinephrin output, must vary with the condition of the heart, and this with the blood flow on which its nutrition depends. Stimulation of the peripheral end of a splanchnic nerve after removal of both adrenals caused also distinct acceleration, as much as 8 to 10 beats per minute, in this experiment although, of course, some further fall of blood pressure had been caused by division of the nerve. Portions of the curves used for counting the heart rate in the last sciatic stimulation prior to removal of the adrenals and the second stimulation after removal are given in figure 12, and a reduction

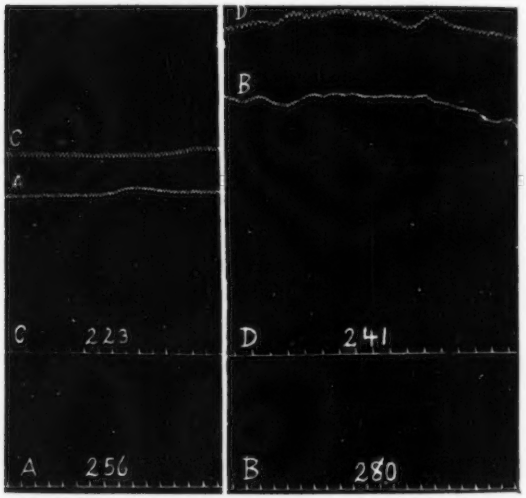

Fig. 12. Parts of blood pressure tracings from cat 449. *A*, before and *B*, a portion commencing 15 seconds after beginning of sciatic stimulation, before excision of both adrenals; *C*, before and *D*, 15 seconds after beginning of sciatic stimulation, after excision of both adrenals. Reduced to four-fifths.

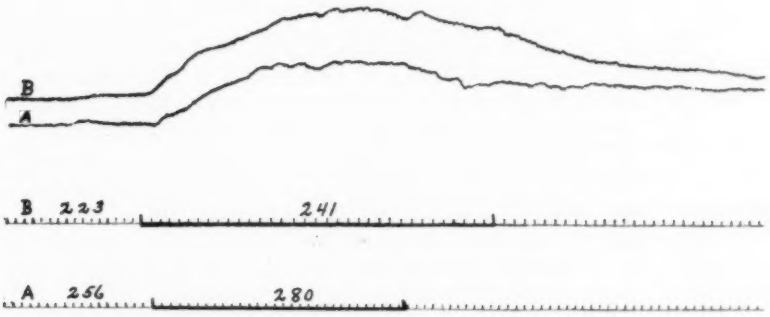

Fig. 13. Blood pressure curves from cat 449. *A*, sciatic stimulation before and *B*, after excision of both adrenals. Reduced to one-half.

of the greater part of the two curves in figure 13. It will be seen that neither the blood pressure reaction nor the acceleration was essentially modified by the absence of the adrenals. The blood pressure, although decidedly lower after the adrenalectomy, was still good (70 to 80 mm. of mercury, as compared with 110 mm. before the operation).

In the next experiment (cat 448) the adrenals were removed extraperitoneally by the lumbar route. The results were practically the same as in cat 449, in which the abdomen had been opened.

Protocol. Cat 448; male; weight, 3.29 kgm. Under urethane (6 gm. in two doses) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
11:43 a.m.	Before sciatic stimulation (10 cm.)	217	132
	10 seconds after beginning stimulation	226	158
	30 seconds after beginning stimulation	232	144
11:45 a.m.	Before sciatic stimulation (8 cm.)	210	135
	10 seconds after beginning stimulation	234	170
	30 seconds after beginning stimulation	242	149
12:20 p.m.	Extirpated both adrenals (extraperitoneally)		
12:25 p.m.	Before sciatic stimulation (8 cm.)	165	90
	6 seconds after beginning stimulation	171	103
	25 seconds after beginning stimulation	170	
12:32 p.m.	Before sciatic stimulation (7 cm.)	164	87
	10 seconds after beginning stimulation	181	132
	26 seconds after beginning stimulation	184	128
	35 seconds after beginning stimulation	182	118
12:40 p.m.	Sciatic now stimulated twice with an interval of only 2 minutes, to fatigue the reaction, and then at		
12:50 p.m.	Before sciatic stimulation (6 cm.)	173	92
	10 seconds after beginning stimulation	181	126
	35 seconds after beginning stimulation	183	
1:03 p.m.	Sciatic again stimulated twice in rapid succession and then at		
1:14 p.m.	Before sciatic stimulation (6 cm.)	171	76
	10 seconds after beginning stimulation	175	107
	40 seconds after beginning stimulation	177	
1:20 p.m.	Opened abdomen, tied renal arteries and veins		
1:24 p.m.	Before sciatic stimulation (6 cm.)	174	88
	4 seconds after beginning stimulation	181	110
	30 seconds after beginning stimulation	181	98
1:33 p.m.	Stimulated sciatic with abdominal aorta clipped.		
	Before clipping aorta	170	77
	Before sciatic stimulation	177	100
	10 seconds after beginning stimulation	184	
	30 seconds after beginning stimulation	186	114
	After release of aorta	184	
	30 seconds after release of aorta	185	86

		Rate	Pressure
1:37 p.m.	Stimulated sciatic with abdominal aorta and vena cava clipped;		
	Before stimulation (7 cm.)	174	76
	8 seconds after beginning stimulation	180	
	30 seconds after beginning stimulation	182	100
	10 seconds after removal of clips from aorta and cava.	176	68
2:21 p.m.	Before sciatic stimulation (7 cm.)	159	47
	10 seconds after beginning stimulation	159	60
	Just after end of stimulation	158	
2:25 p.m.	Before sciatic stimulation (4 cm.)	155	43
	10 seconds after beginning stimulation	161	55
	40 seconds after beginning stimulation	161	

Accelerations as great as 20 beats per minute were obtained after removal of the adrenals. Before removal the maximum acceleration seen in two observations with different strengths of stimulus was 15 and 32 beats respectively. The blood pressure fell from 135 to 90 mm. of mercury after the adrenalectomy. When the sciatic was then repeatedly stimulated with only short intervals between the successive stimulations, the heart reaction, as estimated by the maximum acceleration, diminished but about the same absolute rate (180 to 183) was reached at the height of the acceleration. Later on in the experiment, when the abdomen had been opened and the renal vessels tied, an acceleration of 7 beats per minute was caused by clipping the abdominal aorta just above the bifurcation, as is done in the collection of blood from the cava pocket. This acceleration was accompanied by a rise of blood pressure from 77 to 100 mm. of mercury. When the sciatic was now stimulated with the aorta still clipped (the clip was put on only a short time before stimulation of the sciatic so that the excitation of the nerve should not be interfered with) a further acceleration of 9 beats a minute occurred. Sciatic stimulation gave a similar acceleration with both abdominal aorta and cava clipped, as in making the pocket. At the end of the experiment, when the blood pressure had fallen to 40 to 50 mm. of mercury, no sensible acceleration, or with stronger stimulation only a small one, could be elicited through the sciatic and the rise of blood pressure was also small. But it would surely be absurd to attribute this belated failure of a reaction which had been well obtained after the adrenalectomy to the absence of a reflexly excited outpouring of epinephrin.

As to varying the strength of stimulation, it should be stated that we did not consider there would be any point in merely comparing the reaction obtained before and after the removal of the adrenals with

practically the same nominal strength of stimulus, since the excitability of the reflex mechanisms cannot be counted upon to remain the same, especially where considerable changes of blood pressure have occurred. What we tried to do in all the experiments was to elicit as large a reaction as possible, both before and after elimination of the adrenals, using for this purpose the strength of stimulation which seemed most effective. The strongest stimuli were not generally the best. Not infrequently we found that stimuli of the same strength as gave the maximum acceleration before removal of the adrenals did so after their removal also. But sometimes it was necessary to increase the stim-

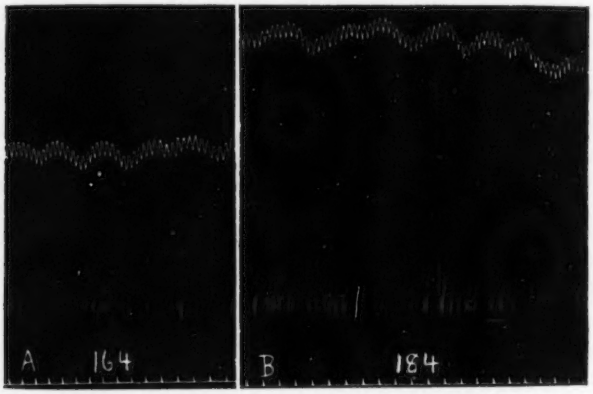

Fig. 14. Parts of blood pressure tracings from cat 448. *A*, before and *B*, a portion commencing 26 seconds after beginning of sciatic stimulation, after excision of both adrenals. Reduced to four-fifths.

ulus. This is mentioned because if the same strength of stimulus is employed before and after removal of the adrenals, a small, or even no acceleration might be obtained after the operation, which would not mean that the reaction had disappeared because of the loss of the adrenals, but that the excitability of the mechanisms concerned in it had diminished. The same is, of course, true of the vasomotor reflex, which we took as an indicator of effective stimulation.

Portions of a curve from cat 448, showing acceleration on sciatic stimulation after removal of the adrenals, are reproduced in figure 14, and the greater part of the curve on a reduced scale in figure 15.

Protocol. Cat 437; female; weight, 1.61 kgm. Left superior cervical ganglion excised one month previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
11:46 a.m.	Before sciatic stimulation[8]	260	116
	5 seconds after beginning stimulation	274	150
11:50 a.m.	Opened abdomen, tied off renal arteries and veins		
11:55 a.m.	Before sciatic stimulation	253	60
	10 seconds after beginning stimulation	264	84
12:00 m.	Before stimulation of sciatic with abdominal aorta clipped	250	64
	2 seconds after beginning stimulation	270	88
	15 seconds after beginning stimulation	273	
12:05 p.m.	Before stimulation of sciatic with abdominal aorta and cava clipped	255	60
	4 seconds after beginning stimulation	278	84
12:10 p.m.	Excised both adrenals		
12:11 p.m.	Before sciatic stimulation	203	44
	4 seconds after beginning stimulation	232	59
2:15 p.m.	Before sciatic stimulation	214	42
	10 seconds after beginning stimulation	225	53

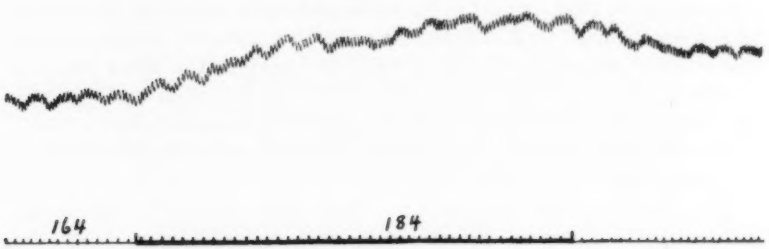

Fig. 15. Blood pressure curve from cat 448. Sciatic stimulation after excision of both adrenals. Reduced to one-half.

In cat 437, in which a superior cervical ganglion had been excised a month previously and in which, therefore, eye reactions were also available, the adrenalectomy was performed at a different stage of the experiment, and after the abdomen had been opened for some time and observations made on the results of sciatic stimulation with the abdominal aorta and cava clipped, as in the collection of blood from the adrenals. The demonstration that the heart reaction is obtained after removal of the adrenals was, if anything, more striking than in many of the other experiments. Sciatic stimulation yielded a maximum

[8] The eye reactions were those described in the footnote to protocol of cat 190, p. 319.

acceleration of 14 beats per minute before the abdomen was opened. After opening the abdomen and tying off the renal vessels, the blood pressure had fallen to 60 mm. of mercury as compared with 116 mm. at the beginning of the experiment. An acceleration of 11 beats was given by stimulation of the sciatic. The abdominal aorta was now clipped and a short interval allowed for any effect on blood pressure and heart rate to develop, and then the sciatic was stimulated. The heart rate compared with that immediately before stimulation was increased by 23 beats per minute. An equal acceleration was given in another observation, in which the abdominal aorta and inferior cava (above the junction of the iliac veins) were clipped in the same way shortly before stimulation. In such observations the clips were not removed till the portion of the curve to be used for counting the heart beats had been completed. Both adrenals were then excised and thereafter an acceleration of 29 beats was caused by stimulation of the sciatic. It is surely impossible to reconcile the results of such an experiment with Cannon's statements: *a*, that after opening the abdomen it is very rare to obtain any acceleration of the heart by sciatic stimulation; *b*, that preparing the cava pocket for collection of adrenal blood, as practised by Biedl, Hoskins and McClure (14), ourselves and other investigators, renders it impossible to detect the great increase in epinephrin output which according to him is indicated by the heart reaction; and *c*, that removal of the adrenals abolishes the reaction because it is due entirely to a reflexly excited increase in the secretion of epinephrin.

The last experiment to which we shall allude (cat 447) was one in which sciatic stimulation caused only a small acceleration at the beginning of the experiment, accompanied by insignificant changes of blood pressure. The cat was only half grown, but it is not known whether this had anything to do with the relatively small effects. The reaction was not essentially modified by removal of the adrenals, or subsequently by clipping the abdominal aorta. The blood pressure was diminished from about 100 mm. to little over 50 mm. of mercury after the adrenalectomy, and the heart rate was also diminished from over 200 to 175 beats a minute. As the blood pressure fell still lower toward the end of the experiment, the heart rate diminished further to 160 beats a minute.

Protocol. Cat 447; female; weight, 1.24 kgm. Under ether cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	*Pressure*
11:00 a.m.	Before sciatic stimulation (8 cm.)	203	115
	Just after end of stimulation	218	110
11:02 a.m.	Before sciatic stimulation (10 cm.)	210	100
	12 seconds after beginning stimulation	207	100
11:05 a.m.	Before sciatic stimulation (9 cm.)	205	110
	10 seconds after beginning stimulation	209	112
11:11 a.m.	Before sciatic stimulation (7 cm.)	206	110
	12 seconds after beginning stimulation	209	118
	20 seconds after beginning stimulation	212	
11:18 a.m.	Before sciatic stimulation (9 cm.)	202	104
	15 seconds after beginning stimulation	208	108
11:40 a.m.	Opened abdomen, excised both adrenals		
11:43 a.m.	Before sciatic stimulation (7 cm.)	175	53
	15 seconds after beginning stimulation	181	63
	40 seconds after beginning stimulation	184	
11:46 a.m.	Before sciatic stimulation (9 cm.)	179	55
	15 seconds after beginning stimulation	182	55
	30 seconds after beginning stimulation	183	55
11:48 a.m.	Before sciatic stimulation (5 cm.)	172	54
	Just after beginning stimulation	177	
	10 seconds after beginning stimulation	172	54
11:53 a.m.	Before stimulation of sciatic with abdominal aorta clipped (8 cm.)	172	55
	6 seconds after beginning stimulation	171	59
	After end of stimulation	179	
12:10 p.m.	Before stimulation of sciatic with abdominal aorta clipped (7 cm.)	160	49
	6 seconds after beginning stimulation	160	51
	25 seconds after beginning stimulation	166	
12:15 p.m.	Before sciatic stimulation (8 cm.)	163	35
	10 seconds after beginning stimulation	166	48
	30 seconds after beginning stimulation	164	

The slowing of the denervated heart after interference with the epinephrin output. It is not easy to demonstrate conclusively that elimination of the epinephrin output of the adrenals causes a slowing of the denervated heart, indicating that the ordinary output, under the conditions of our experiments, is capable of exerting an influence upon the heart. For when the elimination of the epinephrin output is brought about by removal of the adrenals in an acute experiment, the result may be complicated by a slowing associated with a fall of blood pressure. Nevertheless, as stated previously (15), "evidence of a relation of the

normal epinephrin output to the heart rate seems to be afforded by a comparison of the effects produced in cats on the rate by excision of the stellate ganglia after previous section of the vagi when the adrenal epinephrin is normally entering the circulation and in the absence of epinephrin." This is illustrated in table 1.

Although the number of animals is small, the results are suggestive. In the three cats which had been subjected to the adrenal operation mentioned there was a decided diminution in the heart rate after removal of the ganglia. In cat 235 the rate was unusually slow

TABLE 1

CAT	BEFORE		AFTER REMOVAL OF STELLATES		REMARKS
	Rate	Pressure	Rate	Pressure	
231	216	176	(a)152	104	33 days after adrenal operation
			(b)164	82	(b) 10 minutes later than (a)
233	193	87	168	76	36 days after adrenal operation
235	142	106	(a)144	90	34 days after adrenal operation
			(b)129	78	(b) 2 minutes later than (a)
234	266	152	255	122	
236	250	184	(a)249	168	
			(b)233	118	(b) 7 minutes later than (a)
237	192	126	(a)187	110	
			(b)178	104	(b) 4 minutes later than (a)
193	172	120	188	114	Stellates removed an hour after beginning of experiment after repeated splanchnic stimulation
195	181	78	145	52	

In the first three cats the right adrenal had been removed and the nerves of the left cut. The last 5 cats were normal animals. All were anesthetized with urethane.

(142 beats per minute) before the excision of the stellate ganglion and immediately after excision of the second ganglion it was 144. But it at once began to fall and in 2 minutes was 129. An acceleration is not infrequently seen during the operation for removal of the ganglia, due it may be supposed to reflex stimulation of the accelerantes or direct mechanical stimulation or possibly to indirect effects produced through vasomotor changes. The full effect of the elimination of the accelerator nerves may then not be seen for a minute or two.

In cat 231 the epinephrin output as estimated on adrenal vein blood by assay on rabbit intestine segments could not have been one-twenty-

fifth of the normal average. In cat 233 it could not have been more than one-twentieth of the normal average (intestine and pupil assay), and in cat 235 it could not have been one-three-hundredth of the normal; even the adrenal blood sample with the slowest flow (0.6 gm. per minute) was shown by the intestine segment test to have a smaller concentration than 1:300,000,000 adrenalin. In the five normal cats in table 1 only in one was a decided slowing of the heart observed after excision of the stellate ganglia and this was associated with a marked fall of blood pressure.

TABLE 2

CAT	BEFORE		AFTER REMOVAL OF REMAINING ADRENAL		Days after operation
	Rate	Pressure	Rate	Pressure	
441	219	100	214	86	7
444	131	141	137	138	8
450	173	102	174	94	34
438	195	104	(a)195*	74	3
			(b)130	40	
439	210	125	178	54	5
440	226	134	212	124	6
443	180	116	182	83	7
445	161	128	143	86	5
446	185	115	147	57	6
			164	80†	

In the first three cats the right adrenal had been excised and the nerves of the left cut. In cat 445 the left and in the others the right adrenal had been removed. Cats 440, 441, 443, 444, 445 were etherized, the others were under urethane.

* (a) immediately and (b) 4 to 5 minutes after removal of adrenal. The blood pressure was falling continuously during this time.

† After injection of Ringer's solution.

In table 2 are shown the heart rates and blood pressures before and after removal of the remaining adrenal from three cats in which the right adrenal had been excised and the nerves of the left cut, and in seven cats from which one adrenal had been removed at a previous operation. The vago-sympathetics had been cut and the stellate ganglia excised at the beginning of the experiment. In the three cats whose remaining adrenal had been denervated no diminution in the pulse rate was caused by excision of the gland. As already remarked, the fact that the blood pressure was practically unaltered by removal

of the previously denervated adrenal in these cats makes it difficult to estimate the influence of the suppression of the epinephrin output from the gland upon the result. In four out of the other seven cats a substantial slowing of the heart was found after removal of the remaining adrenal. In another of the seven cats (438) although the rate was unchanged immediately after removal of the remaining adrenal the blood pressure was steadily falling and 5 minutes later the pulse rate was only 130.

In table 3 are given the heart rates and blood pressures in five normal cats before and after removal of both adrenals at one operation, the vago-sympathetics having been cut and the stellate ganglia excised at the beginning of the experiment. In every case there was a diminu-

TABLE 3

CAT	BEFORE		AFTER REMOVAL OF BOTH ADRENALS		ANESTHETIC
	Rate	Pressure	Rate	Pressure	
447	203	115	175	53	Ether
448	218	132	165	90	Ether
449	285	125	228	70	Urethane
437	256	116	203	44	Urethane
195*	146	44	131	38	Urethane

* In cat 195 the adrenals were removed about an hour after removal of stellates. The rate just before removal of the adrenals was 145.

tion in the heart rate, but this again was in every case associated with a fall of blood pressure.

Table 4 shows the heart rates and blood pressures in fourteen normal cats after section of the vago-sympathetics and excision of the stellate ganglia. In three dogs anesthetized with morphine and ether the pulse rates after denervation of the heart were 106, 153 and 173, beats per minute, with blood pressures of 92, 72 and 38 mm. of mercury respectively. That different anesthetics may affect the pulse rate of the denervated heart differently may be assumed, e.g., chloroform diminishes the rate in dogs according to v. Anrep (1). So far as can be judged from a relatively small number of observations in our own experiments, the difference between ether and urethane was not conspicuous.

If all the results on the cats are brought together, some further suggestive points seem to emerge. Thus, in twelve out of twenty[9] normal cats with both adrenals intact the pulse rate was over 200 a minute after denervation of the heart. In two out of seven cats with one adrenal previously removed and in only two out of nine cats with one adrenal previously removed and the nerves of the other cut, was the pulse rate over 200 after denervation of the heart. The average pulse rate of the twenty cats with both adrenals intact, after denervation of the heart, was 209 beats per minute; the average for the nine cats whose epinephrin output had been previously interfered with by excision of one adrenal and section of the nerves of the other 170 beats

TABLE 4

CAT	RATE	PRESSURE	CAT	RATE	PRESSURE
201	177	54	190	250	123
202	145	92	191	240	124
215	219	134	196	212	72
175	188		198	233	60
	176	82*	199	137	113
176	160		200	210	84
177	185	132	436	203	56
179	169	80			

* 2 hours after the first observation.

In cats 201, 202 and 215 the right adrenal had been excised and the nerves of the left cut 18 days, 8 days and 59 days respectively before the operation, the others were normal cats. All were anesthetized with urethane, cat 436 more deeply than the rest.

per minute; and the average for the seven cats from which one adrenal had been previously removed 184 beats per minute. A possible influence of the previous operation as such, apart from interference with epinephrin output, where the interval was only a few days is not excluded, but is not discernible in the tables.

[9] In 4 additional normal cats the heart rates and blood pressures after denervation of the heart, but before adrenalectomy, were 266 (214), 284 (145), 265 (145), and 150 (66). In the last cat the abdomen had been opened before the heart was denervated. After removal or ligation of both adrenals the corresponding numbers were 222 (114), 283 (102), 255 (130), and 143 (57). In a fifth cat the rate after denervation of the heart was 248 and the blood pressure 150 mm. of mercury.

DISCUSSION AND SUMMARY

It has been shown by us that the acceleration of the heart caused by stimulation of the central end of the sciatic is in no way a reaction by which the rate of output of epinephrin from the adrenals can be estimated, or changes in that rate demonstrated, as claimed by Cannon. This is proved by the following facts:

a. Clipping of the adrenal veins has no demonstrable influence upon the occurrence and magnitude of the heart reaction caused by sciatic stimulation, although it markedly diminishes or abolishes reactions which are known to be genuine reactions for epinephrin, such as the dilatation of the pupil following stimulation of the peripheral end of a splanchnic nerve.

b. Acceleration of the denervated heart on sciatic stimulation is well obtained in cats which have been allowed to survive after removal of one adrenal and section of the nerves of the other, an operation which is known to abolish or greatly diminish the epinephrin output. In such cats the reaction is still elicited after the remaining adrenal has been removed.

c. Good acceleration of the heart can be elicited by stimulation of the sciatic in cats from which both adrenals have been removed, either in two operations with an interval for recovery interposed, or at one operation.

d. When the reaction disappears after removal of the adrenals this is not because of the absence of increased epinephrin discharge on stimulation of the sciatic but for other reasons, such as deterioration in the condition of the animal (fall of blood pressure, etc.) which interferes with the reflex or reflexes necessarily involved in the reaction or with the capacity of the heart to markedly accelerate its beat.

e. Contrary to Cannon's statement, the reaction is well obtained after opening the abdomen. It can be elicited after ligation of the renal vessels, abdominal aorta and inferior cava, as practised in forming a cava pocket for collection of adrenal vein blood. If the reaction indicates a greatly increased output of epinephrin reflexly induced by stimulation of the sciatic, as assumed by Cannon, we could not have failed to detect the increase by the direct method of collecting adrenal vein blood and assaying its epinephrin content on rabbit segments. But our results were negative (16).

As regards the real mechanism of the acceleration of the denervated heart caused by sciatic stimulation, we desire to point out, once for

all, that the onus of explaining this probably complex indirect reaction, which Cannon erroneously interprets as indicating increased epinephrin secretion, does not rest upon us at all. It is for Doctor Cannon to exclude, if he can, by control experiments, other possible factors in the reaction which he attributes solely to epinephrin. Our position is simply this. We have investigated the influence of stimulation of the sciatic and brachial nerves upon the rate of epinephrin output by a direct method, correct in principle and free from ambiguity, and have obtained negative results. Doctor Cannon states that by means of an indirect method (the denervated heart reaction) he obtains a positive result. We show that this reaction cannot yield any information as to the rate of epinephrin output from the adrenals or as to changes in that rate, since it is obtainable when the epinephrin output of the adrenals is abolished. And here we are entitled to rest our case, not, of course, claiming that sensory stimulation *cannot* increase the epinephrin output, but that no increase has hitherto been proved.

However, certain fairly obvious suggestions may be made as to factors which may play a part in the heart reaction under discussion, a reaction probably made up of more than one component. One is the larger amount of epinephrin sent through the coronary circulation and perhaps the greater concentration of it, owing to the vasomotor changes produced by stimulation of the sciatic (11), (15). It may be pointed out that even if Cannon's statement that the reaction cannot be obtained in the absence of the adrenals had been found correct, that of itself would only have shown that epinephrin is essentially concerned. For it might be due to the redistribution of the epinephrin without any increase in the rate of output.

Cannon attempts to invalidate this suggestion by an experiment in which he prevents a rise of pressure in the carotid during sciatic stimulation by compression of the chest, and yet obtains an acceleration of the heart. Now this is a quite complex experiment, and Doctor Cannon is doing several other things which may affect the heart besides keeping the pressure in the aorta constant. One of the things he is doing is impeding the venous return. The epinephrin, even if its rate of output remains unchanged, must, therefore, be diluted with a smaller proportion of indifferent blood in the right heart. Blood with a greater concentration of epinephrin must accordingly be passing through the coronaries, and as the concentration will increase in the same measure as the slowing in the venous return necessary to prevent rise of pressure, approximately the same amount of epinephrin will pass through the coronaries per unit of time as with a similar sciatic stimulation without compression of the chest. If then the ordinary output of epinephrin was a factor in the acceleration without compression it may be expected to exert the same influence during compression. In other words, an increase in the amount of epinephrin passing per unit of time through the coronary circulation during sciatic stimulation is not prevented by compressing the chest, so as to keep the blood pressure in the aorta from rising,

even if no increase in the rate of output of epinephrin has occurred, and the experiment is without significance for the question at issue. That no further acceleration occurred when the blood pressure rose after releasing the chest, is also just as intelligible, so far as epinephrin is a factor, on the assumption that the epinephrin output was not increased as on the assumption that it was increased by stimulation of the sciatic. For if the epinephrin concentration remained unchanged and the coronary blood flow was increased on decompressing the chest, an increased amount of epinephrin would pass through the coronaries per minute whether the output had been augmented by stimulation of the sciatic or not. The fact is, however, that with the increase in the venous inflow to the heart the concentration of epinephrin in the blood of the right heart must be proportionally diminished. But why, in any case, should a further increase in the heart rate have been expected, since the acceleration was already 36 beats a minute, the same as without compression of the chest? As there is no possibility that epinephrin is the sole factor in the heart reaction, as elicited reflexly, the point need not be labored. Whatever other factors are ordinarily concerned in the reaction, apart from the rise of arterial blood pressure, may be expected to act as well during compression of the chest as before. The possibility that a special factor, the gross interference with the mechanism of the heart, especially the filling and pressure of the right side and with the respiration might exert an influence in this experiment is not excluded.

We ourselves made two experiments (cats 198, 199) in which the rise of pressure on sciatic stimulation was largely prevented by hemorrhage, controlled by a mercury valve. The animals were anesthetized with urethane. In cat 198 before sciatic stimulation the pulse was 225, the blood pressure 78; during stimulation the pulse rate rose to 253 and the blood pressure to 192 mm. of mercury. In the next observation the pulse was 233 and the blood pressure 60 before stimulation. During stimulation the blood pressure was prevented from rising beyond 90 mm. of mercury, and the pulse rate increased only to 242 beats per minute. Blood (mixed with salt solution) was reinjected. Before stimulation of the sciatic the pulse rate was 272, the pressure 96. During stimulation the pulse rate rose to 286 with a pressure of 168 mm. of mercury. The sciatic was now stimulated while the pressure was prevented, by hemorrhage, from changing (it rose from 88 to 92). The pulse rate before stimulation was 258 and during stimulation 259. This experiment might seem to show that the acceleration was largely prevented by keeping the blood pressure from rising. But in the other cat a different result was obtained. In cat 199 the following pulse rates and blood pressures, the latter in parentheses, were recorded. Before stimulation 137 (113), during stimulation 215 (196). Before stimulation 130 (108), during stimulation with hemorrhage 193 (116–90). Before stimulation 144 (100), during stimulation 206 (162). Before stimulation 145 (102), during stimulation with hemorrhage 194 (115). Only these two experiments were made. For on reflection it was seen that this method also could not lead to any definite conclusion. If the epinephrin were the only factor in the acceleration, its concentration in the blood coming to the heart would increase as the mass of the circulating blood diminished, even provided that no increase were taking place in the rate of output.

Besides the direct accelerating action of epinephrin upon the heart there is another way in which the normal output of epinephrin may possibly play a part

in the acceleration caused by stimulation of the sciatic, by sensitizing the heart to the action of other factors, such as a rise of blood pressure, which in the absence of epinephrin might not be so effective. In this connection we recall the observation of v. Anrep (1) on the influence of adrenalin upon the power of the heart to adapt itself by changes in its tone to changes in the arterial pressure.

Whatever share epinephrin may take in the acceleration reaction it cannot be the only factor and is probably not the most important one, since excellent heart reactions can be obtained in the absence of the adrenals, provided that good vascular reflexes, as evidenced by the change of blood pressure, are elicited. The most obvious of the changes caused by stimulation of the central end of the sciatic, the rise of blood pressure, is the one which seems to be most intimately related to the heart acceleration. Since this relation is seen after, as before, elimination of the adrenals, the most direct suggestion is that the better blood flow through the coronary circulation is an important factor in the acceleration, either by raising the nutritive condition and the excitability of the mechanism in which the beat originates or by acting upon a local accelerator mechanism. An action of the increased blood pressure as such is not excluded. Indeed, long ago Johansson (17) pointed out that the acceleration of the heart (after section of the vago-sympathetics and excision of the stellate ganglia) caused by stimulation of the peripheral end of a splanchnic nerve and of the cut cervical cord, was dependent on the abruptness of the rise of pressure. At the time of Johansson's work, nothing was known of the secretory innervation of the adrenals, and it might be asked whether the whole acceleration in his experiments was not due to increased epinephrin output. This question, in our opinion, must be answered in the negative. For stimulation of the cord with one splanchnic cut produced in general a much greater acceleration than stimulation of one splanchnic, without any obvious reason why it should have caused a greater liberation of epinephrin, and this greater acceleration was accompanied by a larger rise of blood pressure. Further the dogs were curarized and curara (18) depresses the conductivity of the efferent epinephrin secretory path. We do not, however, know how much importance should be attached to this, as our work with curara was done on cats and was concerned only with the spontaneous liberation of epinephrin.

It is significant that the accelerations observed by him, taken in relation to the blood pressure changes, are of the same order of magnitude as those obtained by us with splanchnic and sciatic stimulation, whether with the adrenal veins clipped or in the absence of the adrenals. From our own observations, recorded in the preceding pages, it cannot be doubted that a rise of pressure, caused by stimulation either of the sciatic or the peripheral end of the splanchnic nerve, is associated with an acceleration of the denervated heart, unrelated to any immediate action of epinephrin. The maximum acceleration, as in Johansson's observations, may not be reached till the blood pressure has again begun to decline.

We agree with Johansson and with Lehndorff (19) that the manner in which the rise of pressure is produced is important as regards its action upon the heart. The latter observer remarks that raising the pressure by compressing the aorta seldom causes an acceleration, and that when acceleration is caused it is only slight. He was never able by inducing an asphyxial rise of pressure to elicit

the characteristic changes in the heart's action which he found on splanchnic stimulation. Guthrie and Pike (20) also found compression of the aorta inefficient. We have seen a definite acceleration, e.g., in cat 190 (see protocol), clipping the aorta in the thorax increased the pulse rate from 249 to 260 beats a minute, but this was a much smaller acceleration than that caused by splanchnic stimulation just before, although the increase of arterial pressure was greater when the aorta was clipped. In other cases we have seen no acceleration when the aorta was clipped in the thorax. Clipping of the abdominal aorta has been sometimes seen to cause some acceleration with a moderate rise of pressure in the absence of the adrenals. The character of the blood sent to the heart is, not the same when the thoracic as when the abdominal aorta at the bifurcation is clipped, the liver and other viscera not being interfered with in the latter case. Apart altogether from the possible effect of epinephrin when the splanchnic is stimulated, the mechanical conditions under which the heart works are very different when the aorta is clipped and when the central end of the sciatic or the peripheral end of the splanchnic is stimulated, particularly as regards the venous inflow.

Cannon seems to criticise us for quoting the experiments of Guthrie and Pike on the influence of increased pressure of the perfusion fluid in accelerating the excised heart, as if this represented the sum total of our knowledge. We quoted this work, which was done in the laboratory of one of us, in a footnote to another paper (11), to show, and we believe it does show, that under certain conditions the heart, deprived of extrinsic innervation, can respond to changes of blood pressure by very considerable changes in frequency. When we stated that there is nothing strange about an increase in the rate of the denervated heart *in situ* when the central end of the sciatic or the peripheral end of the splanchnic is stimulated, and went on to say that it is obviously dependent upon the better blood flow through the coronary vessels, we had before us much of the evidence given on preceding pages that marked acceleration could be produced by stimulation of both of these nerves, in the absence of any possible output of epinephrin from the adrenals, and that the acceleration was associated with a rise of arterial pressure. Of the results of other observers on the heart *in situ*, we had in mind particularly the elaborate paper of Johansson (17) already referred to. The experiments of Martin and of Knowlton and Starling on the heart-lung preparation, which Cannon cites, were familiar to us, but we saw no point in quoting in a footnote observations which threw no light upon the accelerations we were obtaining by stimulation of the sciatic, under conditions which eliminated the liberation of epinephrin from the adrenals. Of course, the vasomotor reactions expressed in the rise of blood pressure under discussion might possibly have other actions upon the denervated heart, in addition to their most obvious action, the increased blood flow through the coronaries. It has been pointed out by various writers that it is not the same thing, as regards the heart rate, whether changes are induced in the arterial or in the venous pressure.

In the case of sciatic stimulation it might still be argued that the rise of pressure is only a sign that the stimulus is effective for some other reflex or reflexes on which the acceleration depends essentially. This is theoretically true, but any reflex which affects the composition of the blood, as sciatic stimulation may do (by causing hyperpnoea, increased reflex muscular action, possibly increased

mobilization of sugar or other changes in the liver through the splanchnics), must affect the heart concomitantly with the vasomotor reflex which increases the blood flow through the coronaries. In any case, the experiments on stimulation of the splanchnic in the absence of epinephrin output indicate the rise of arterial pressure as the most obvious of the factors associated with the heart reaction studied.

In two cats (one normal, 447, and one in which the epinephrin output had been interfered with, 444, both etherized), we have seen an acceleration, when sciatic stimulation elicited no rise of pressure either before or after elimination of the adrenals. But then there was evidence of effective stimulation, not only in the increased respiratory movements and respiratory blood pressure waves but also in a small depression of the blood pressure, succeeded by some rise after stoppage of the stimulation. In such a case the question presents itself, whether it is certain that in every individual the heart is completely severed from the central nervous system by section of the vagi and excision of the stellate ganglia. We repeat that, having shown, as we believe, that Cannon's supposed proof, by means of the heart reaction, of augmented epinephrin output through stimulation of the sciatic is illusory, we do not consider that the explanation of the mechanism of the reaction is our concern. We have simply made some suggestions. Nor do we judge it necessary to discuss in this paper his supposed proof of the augmenting action of asphyxia and of emotional excitement upon the output. Clearly all his conclusions upon this matter stand or fall together. It may be mentioned, however, that we have observed acceleration of the heart, induced by asphyxia, after removal of the adrenals.

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THE EFFECT OF ACIDS, ALKALIES AND SALTS ON CATALASE PRODUCTION

W. E. BURGE

From the Physiological Laboratory of the University of Illinois

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The acids used were hydrochloric, propionic, acetic and butyric; the alkalies or alkaline salts, sodium carbonate, sodium phosphate, ammonium carbonate and sodium acetate; the neutral salts, disodium phosphate, ammonium chloride; and the acid salt, monosodium phosphate. The animals were rabbits and dogs. The method of administration and the amounts of the substances used will be given in the description of the experiments. The catalase of the blood was determined before as well as at intervals after introducing the materials into the alimentary tract of the animal. The determinations were made by adding 1 cc. of blood to hydrogen peroxide in a bottle and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the cubic centimeter of blood. The dog's blood was used undiluted while the rabbit's blood was diluted 1 to 5 with 0.9 per cent sodium chloride. The effect on the catalase of the jugular blood of the introduction of the acids, alkalies and salts into the alimentary tract of rabbits and dogs is shown in figure 1. The figures along the ordinates represent percentage increase or decrease in catalase and those along the abscissae time in minutes.

It may be seen under *acids* that the introduction of 1.5 gram per kilo of acetic acid dissolved in 75 cc. of water per kilo into the stomach of rabbits increased the catalase of the blood 22 per cent in one rabbit and 12 per cent in another in 90 minutes; proprionic acid increased the blood catalase 16 per cent; butyric, 10 per cent; that 1.5 gram per kilo of hydrochloric acid dissolved in 75 cc. of water per kilo decreased the blood catalase 7 per cent in one rabbit, 9 per cent in another rabbit; and that 3 grams per kilo of hydrochloric acid decreased the catalase 28 per cent. While these percentages are in most cases small, at the same time they represent large differences in the amount of oxygen liberated by the blood from hydrogen peroxide. An increase or decrease

of 20 per cent in catalase, for example, represents an increase or decrease of about 100 cc. of oxygen liberated.

It may be seen further in figure 1 under *carbonates*, *phosphates* and *sodium acetate* that the introduction of these substances into the upper part of the intestine of dogs produced an increase in the catalase of the blood of the jugular. The amounts of these salts used were 10 grams per kilo dissolved in 75 cc. of water per kilo, with the exception of

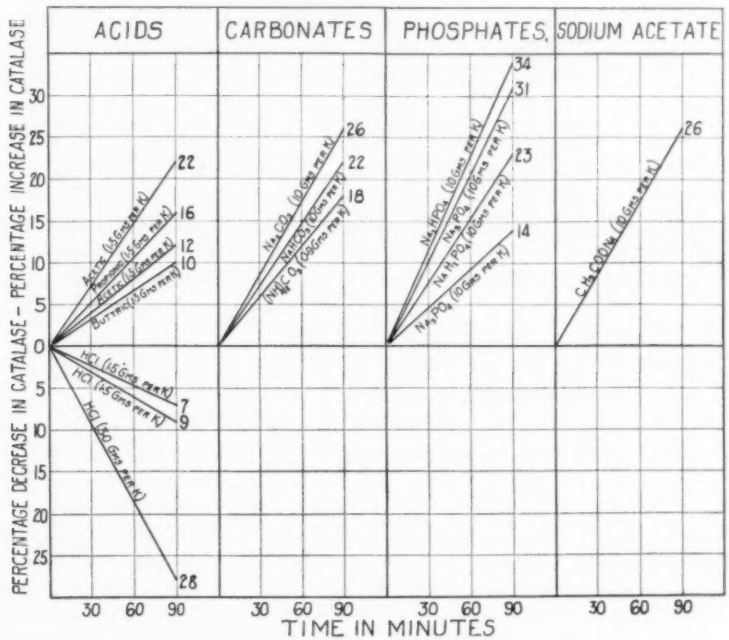

Fig. 1. Curves showing effect in vivo of the substances named in the chart on the catalase content of the jugular blood.

ammonium carbonate, 0.9 gram per kilo of this salt being used. After opening the abdominal wall of dogs while under ether, the materials were injected into the upper part of the small intestine. The wound was sewed up and the ether discontinued after the introduction of the substances.

The question that arises in this connection is, how is the increase as well as the decrease in catalase, observed in the preceding experi-

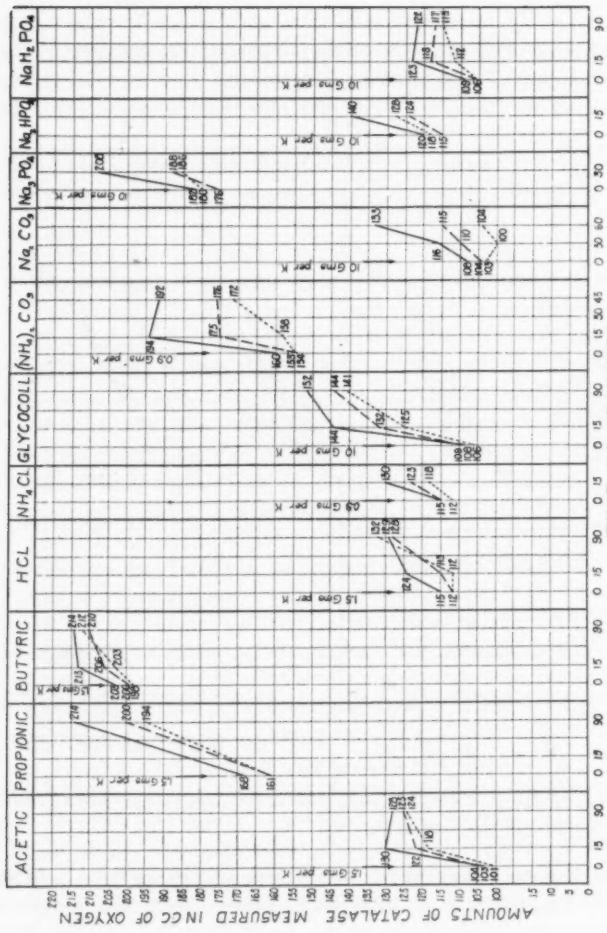

Fig. 2. Curves showing effect in vivo of the substances named in the chart on the catalase contentof the blood. The continuous line curves show amount of catalase in the blood of the liver, the dash line curves the amount in the blood of the portal vein, and the dotted line curves the amount in the blood of the jugular vein.

ments, brought about. We (1) had already found that the ingestion of food, for example, produced an increase in catalase by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme, and that narcotics produced a decrease in catalase by decreasing its output from the liver and by direct destruction. So we naturally turned to the alimentary glands, particularly the liver, in looking for an explanation of the change in the amount of the blood catalase observed in the preceding experiments.

After opening the abdominal wall of a dog while under ether, catalase determinations were made using 1 cc. of blood from the liver, portal and jugular veins. The material to be studied was then introduced into the intestine and catalase determinations were made at fixed intervals. The results of the determinations are given in figure 2. The figures along the ordinate indicate amounts of oxygen liberated from hydrogen peroxide in 10 minutes by 1 cc. of blood, and the figures along the abscissae time in minutes. The continuous line curves were constructed from data obtained from the blood of the liver, the dash line curves from the blood of the portal, and the dotted line curves from the blood of the jugular vein.

Under *acetic* it may be seen that previous to the introduction into the intestine of a dog of 1.5 gram per kilo of acetic acid, dissolved in 75 cc. of water per kilo, 1 cc. of blood from the liver liberated 103 cc. of oxygen in 10 minutes from hydrogen peroxide; 1 cc. of blood from the portal liberated 104 cc. of oxygen, and 1 cc. from the jugular 101 cc. of oxygen. Fifteen minutes after the introduction of the acetic acid, the liver blood liberated 130 cc. of oxygen, the portal blood 122 cc. and the jugular blood 118 cc. Similarly, it may be seen that 90 minutes after the introduction of the acetic acid the liver blood liberated 128 cc. of oxygen, the portal blood 125 cc. and the jugular 124 cc.

By comparing these figures it may be seen that the liver blood, particularly during the first fifteen minutes after the introduction of the acetic acid, liberated more oxygen from hydrogen peroxide than did the portal blood, and that this blood in turn liberated more oxygen than did the jugular blood. This is taken to mean that the acetic acid was stimulating the alimentary glands, particularly the liver, to an increased output of catalase. It may be seen further that proprionic, butyric and hydrochloric acids, ammonium chloride, glycocoll, ammonium carbonate, sodium carbonate and the phosphates produced an increase in catalase in a similar manner, namely, by stimulating the liver to an increased production of this enzyme.

The question that arises in this connection is, does the increase or decrease in the amount of oxygen liberated in the preceding experiments represent an actual increase or decrease in catalase, or does it simply represent a change in catalytic activity brought about by a decrease in the acidity of the hydrogen peroxide. So far as I have been able to find, Jacobson (2) was the first to show that catalytic activity is greatly decreased by acids. This work has been repeated and confirmed by several investigators (Loevenhart (3); Issajew (4); Senter (5); Winternitz and Rogers (6); Mendel and Leavonworth (7); Bodansky (8)) and extended to include the effect of acid salts, alkalies and alkaline salts on catalytic activity. The description of a simple experiment will suffice to show the effect of acids and alkalies on catalytic activity. If catalase determinations are made using equal quantities (1 cc.) of blood and neutral, slightly acid and slightly alkaline hydrogen peroxide, it will be found that the amount of oxygen liberated from the neutral and slightly alkaline hydrogen peroxide will be the same, while that liberated from the slightly acid hydrogen peroxide will be less. By increasing the acidity of the hydrogen peroxide, the amount of oxygen liberated will be decreased more and more until the action of the catalase is completely inhibited, and no oxygen is liberated. By the addition of an alkali (sodium hydroxide) to the acid hydrogen peroxide, the amount of oxygen liberated will be increased until a certain maximum is reached. Further addition of alkali at this point will begin to decrease the amount of oxygen liberated. It should be mentioned in this connection that alkalies are not nearly so effective in decreasing catalytic activity as the acids. Hence the amount of oxygen liberated from acid hydrogen peroxide can be increased by simply neutralizing the acid of the hydrogen peroxide by means of an alkali, or it can be decreased by making the hydrogen peroxide more acid.

Since in our experiments acids and alkalies were used, it might be thought by some that what we have been observing is not an increase or decrease in catalase, but only a change in catalytic activity due to an increase or decrease in the acidity of the hydrogen peroxide. The following experiments were carried out in an attempt to clear up this phase of the problem. It should be said in this connection that most of these experiments had already been performed time and again in connection with our previous work on catalase, but they were not thought of sufficient importance to publish.

Twenty-five cubic centimeters of blood were taken from the external jugular of a dog and defibrinated. It was found that defibrination had

little or no effect on the catalase content of the blood. The abdominal wall of the dog was opened while the dog was under ether and 10 grams per kilo of sodium carbonate dissolved in 75 cc. of water per kilo were introduced into the upper part of the small intestine. The wound was then sewed up and the ether discontinued. Ninety minutes later 25 cc. more of blood were drawn from the jugular and defibrinated. The blood drawn before the introduction of the carbonate will be referred to hereafter as normal blood, while that drawn 90 minutes after the introduction of the carbonate will be referred to as carbonate blood. The catalase content of 1 cc. of the normal blood and of the carbonate blood was determined using slightly acid hydrogen peroxide, and it was found that the carbonate blood liberated about 30 per cent more oxygen than the normal blood. If this increase in the amount of oxygen liberated by the carbonate blood be due to the neutralization of the acid of the peroxide, thus removing the inhibiting effect of the acid, then the difference in the amount of oxygen liberated should be greatly decreased or disappear when neutral or slightly alkaline hydrogen peroxide is used. Catalase determinations of this same sample of defibrinated dog's blood were accordingly made, using neutral and slightly alkaline hydrogen peroxide. It was found that the carbonate blood liberated about 24 per cent more oxygen than the normal blood. This experiment would seem to show that only a very small per cent (6 per cent) of the increase in oxygen liberated by the carbonate blood was due to the neutralizing effect of the carbonate blood, while the remaining 24 per cent was due to an actual increase in catalase.

Further experiments bearing on this point of the following kind were carried out. Two cubic centimeters of blood were taken from the jugular of a dog. The catalase content of 1 cc. was determined immediately, while the other cubic centimeter was shaken with 10 cc. of 0.9 per cent of sodium chloride and placed in a water bath at 80°C. until the enzyme was destroyed. This tube was shaken at intervals to prevent the formation of a firm coagulum. Ten grams per kilo of sodium carbonate were introduced into the intestine of this animal and 90 minutes later another 2 cc. of blood were drawn from the jugular. The catalase of 1 cc. of this was determined immediately, while the other cubic centimeter was added to salt solution and heated at 80°C. for the same time that the control had been. The amount of oxygen liberated from hydrogen peroxide by the cubic centimeter of normal blood was 120 cc., while the carbonate blood liberated 168 cc. The heated cubic centimeter of normal blood as well as the heated cubic

centimeter of carbonate blood was added to slightly acid hydrogen peroxide. One cubic centimeter of blood from the same sample was added to each of these bottles and the amount of oxygen liberated was determined. Slightly more oxygen was liberated from the peroxide containing the heated carbonate blood than from that containing the heated normal blood. This result was the same as was obtained in the preceding experiment in that it shows that the neutralizing effect of the carbonate blood is responsible for a very small part of the increase in oxygen liberated by the blood after the introduction of sodium carbonate into the alimentary tract when slightly acid hydrogen peroxide is used. It should be mentioned in this connection that slightly acid hydrogen peroxide was not used in the experiments on the effect of acids, alkalies and salts reported in this paper, but neutral or slightly alkaline hydrogen peroxide.

Ten cubic centimeters of dog's blood were drawn from the jugular before as well as 90 minutes after the introduction of sodium carbonate into the alimentary tract and defibrinated. The carbonate blood liberated about 18 per cent more oxygen than the normal blood. Both of these samples were introduced into dialyzing tubes made of collodion and dialyzed against 2 liters of 0.9 per cent sodium chloride for 18 hours. At the end of this time these two samples were removed from the dialyzing tubes and made equal in amount by the addition of 0.9 per cent sodium chloride. Catalase determinations were made using 1 cc. from each of these samples, and it was found that dialyzing decreased very slightly the difference in the catalytic activity of the two samples. It had been previously found that catalase did not dialyze through these membranes to any appreciable extent. If the increase in the catalytic activity of the carbonate blood were due to the presence of the alkali in the blood, it would seem that dialyzing should have done away with the difference in activity between the normal and the carbonate blood or greatly decreased it. It might be objected, however, that the carbonate in the blood was in some way fixed and rendered non-dialyzable. Hence, the following experiments were carried out to determine if this objection were valid.

Ten cubic centimeters of blood were drawn from the jugular before as well as after the introduction of 10 grams per kilo of sodium carbonate into the intestine of a dog. It was found that the carbonate blood liberated 16 per cent more oxygen than the normal blood from neutral hydrogen peroxide. These two samples of blood were ashed at a temperature too low to decompose sodium carbonate, and the ash of

each was introduced into bottles containing equal quantities of neutral hydrogen peroxide. One cubic centimeter of blood taken from the same sample was added to each of these bottles and the amount of oxygen liberated from each was determined. It was found that the bottle containing the ash of the carbonate blood liberated practically the same amount of oxygen as that containing the ash of the normal blood.

If the increase in catalase after the introduction of alkalies into the intestine were due to the neutralizing effect on the acid of the hydrogen peroxide, then the introduction of acids into the alimentary tract should decrease the catalytic activity of the blood. In figure 2 it may be seen that the introduction of acids as well as acid salts increased the catalase of the blood instead of decreasing it. Furthermore, if alkalies when introduced into the alimentary tract of an animal increase catalytic activity by neutralizing the acid of the hydrogen peroxide, then the catalytic activity of the blood of the portal should be increased parallel with that of the liver. In figure 2 it may be seen under *ammonium carbonate*, *sodium carbonate* and *sodium phosphate* that such is not the case, but that the catalase of the liver blood is increased much more rapidly than that of the portal. This was interpreted to mean that these substances produced an increase in catalase by stimulating the liver to an increased output of this enzyme.

Determinations similar to the preceding were carried out using sodium phosphate instead of sodium carbonate with practically the same results. From these experiments the conclusion may be drawn that while a very small percentage of the increase in the power of the blood to decompose hydrogen peroxide after the introduction of alkalies and alkaline salts into the alimentary tract of animals is due to an increase in the catalytic activity when acid hydrogen peroxide is used, by far the larger part of the increase is due to an actual increase in catalase brought about by the stimulation of the alimentary glands to an increased output of this enzyme. It might be well to mention again in this connection that neutral or slightly alkaline hydrogen peroxide was used in the experiments reported in this paper on the effect of acids, alkalies and salts on the blood catalase, hence none of the oxygen liberated in these experiments can be attributed to the neutralizing effect of the alkalies.

Chvostek (9) showed that when hydrochloric acid was administered to a rabbit it decreased oxidation, while Lehmann (10) showed that an alkali, such as sodium carbonate, increased oxidation. In figure 1 it may be seen that the administration of hydrochloric acid to rabbits

produced a decrease in catalase, which is offered in explanation for the decreased oxidation as observed by Chvostek. The following experiment will show that the decrease in the blood catalase after the administration of hydrochloric acid to the rabbit is due in part to the inhibiting effect of the acid on the catalase, and in part to the direct destruction of the enzyme.

Ten cubic centimeters of blood were drawn from the jugular of a large rabbit and defibrinated. Two grams per kilo of hydrochloric acid dissolved in 75 cc. of water per kilo were introduced into the stomach of the animal. Ninety minutes later another 10 cc. of blood were drawn and defibrinated. The blood taken before the introduction of the acid will be referred to hereafter as normal blood and that after the introduction of the acid as acid blood. Catalase determinations were made of these two samples of blood and it was found that the acid blood liberated about 15 per cent less oxygen than the normal blood. One cubic centimeter of each of the samples was added to tubes containing 10 cc. of 0.9 per cent sodium chloride and heated at 80°C. until the catalase was destroyed. The heated normal blood as well as the heated acid blood was added to neutral or slightly acid hydrogen peroxide and catalase determinations were made using the normal blood. It was found that the bottle containing the heated acid blood liberated 10 per cent less oxygen than that liberated by the bottle containing the heated normal blood. This is interpreted to mean that of the 15 per cent decrease produced by the introduction of hydrochloric acid into the stomach of the rabbit, 10 per cent was due to the inhibiting action of the acid while the remaining 5 per cent was due to the direct destruction of the enzyme.

In figure 2 it may be seen that the introduction of hydrochloric acid into the intestine of a dog increased the blood catalase instead of decreasing it, as was found to be the case with the rabbit in figure 1. It may be seen further in figure 2 that ammonium chloride is a very effective stimulant to catalase production, 0.9 per cent per kilo being sufficient to bring about a great increase in this enzyme. Since the dog is a carnivorous animal, a relatively large amount of ammonium chloride is formed, when hydrochloric acid is administered, in the neutralization of this acid by ammonia, whereas the rabbit being a herbivorous animal, a relatively small amount is formed. Hence the increase in catalase after the introduction of hydrochloric acid into the intestine of a dog is attributed to the stimulating effect of the ammonium chloride on the liver giving an increased output of this enzyme.

Grafe (11) found that ammonium chloride increased oxidation in the rabbit. Lusk (12) showed that the ingestion of an amino acid, such as glycocoll, increased oxidation in the dog. The increase in catalase with resulting increase in oxidation after the ingestion of glycocoll is attributed to the stimulation of the liver by the glycocoll as well as by the ammonium carbonate and acetic acid resulting from the deamination of the glycocoll.

Further evidence that catalase is probably involved in the oxidative processes is found in the following observations. Müller-Thurgau (13) found that the respiratory metabolism is greatly decreased in potatoes kept for several days at 0°C., that it is greater at the beginning than at the end of the rest period, and also that it is greater in large mature potatoes than in small immature potatoes. Appleman (14) found that there was an increase in catalase in potatoes under the same conditions that Müller-Thurgau had found an increase in the respiratory metabolism and a decrease in catalase where he had found a decrease in metabolism. Appleman also found that there occurred an increase in catalase in the greening as well as the sprouting of potatoes parallel with the increase in the respiratory metabolism, while the oxidases were not increased and, in fact, were slightly decreased.

Hasselbalch (15) found that oxidation or metabolism is very low during the first month of life, and Magnus-Levy and Falk (16) that it is high during childhood. As a result of the work of these three observers and of Bailey and Murlin (17), Murlin and Hoobler (18), Howland (19), Benedict and Talbot (20), Benedict, Emmes, Roth and Smith (21), Palmer, Means and Gamble (22) and others, it is now considered that oxidation or metabolism is low during the first month of life, high during childhood and low after the onset of old age. Battelli and Stearn (23) found that the catalase content of most of the tissues and particularly of the liver of newly-born pigs is lower than the corresponding tissues of the mother, but that the catalase activity rapidly increased until at the end of the seventh or eighth day it was as high as that of the adult. We repeated and confirmed these observations, using the dog and newly-born puppies. We also determined the catalase content of the tissues of puppies that were about ten weeks old and found that the tissues generally were richer in catalase than those of the mother. The catalytic activity of the liver, for example, of the ten-week-old puppies was about 30 per cent greater than that of the liver of the mother. The catalase was determined by adding 1 gram of the hashed tissue, that had been washed free of blood to hydrogen peroxide and

the amount of oxygen liberated in 10 minutes was taken as a measure of the amount of catalase.

Warburg (24) found that during the process of fertilization, oxidation was greatly increased in the sea-urchin egg. Winternitz and Rogers (25) showed that the unfertilized hen's egg showed no catalytic activity even after prolonged incubation, whereas the incubated fertilized egg rapidly acquired the power of decomposing hydrogen peroxide. Doubtless if Winternitz and Rogers had determined the intensity of oxidation in the fertilized hen's egg, they would have found that it increased parallel with the increase they observed in catalase, and if Warburg had determined the catalase content of the fertilized sea-urchin egg, he would have found that this enzyme increased parallel with the increase he observed in oxidation.

J. Loeb (26) attributes the development of the fertilized egg to the increase in oxidation, and the increase in oxidation to a change in the cortex of the egg which makes the entrance of oxygen, and hence oxidation, possible, while R. Lillie (27) holds that the cortical layer of the unfertilized egg prevents the diffusion of carbon dioxide from the egg and that this carbon dioxide prevents oxidation and hence development. A more plausible explanation for the increased oxidation or metabolism in the fertilized egg, and hence for the development of the egg, would seem to be that the spermatazoön furnishes a substance which stimulates the egg to an increased formation of catalase. Further evidence in support of this view is afforded by the fact that the very same chemicals (amines, alkalies, acetates, butyric acid, etc.) which Loeb found would bring about increased oxidation and artificial parthenogenetic development of the egg, we found, when introduced into the alimentary tract of animals, stimulated the alimentary glands, particularly the liver, to an increased output of catalase with resulting increase in oxidation.

SUMMARY

1. The increase in oxidation produced by the introduction of an alkali such as sodium carbonate or phosphate into the alimentary tract of an animal is attributed to the increase in catalase brought about by the stimulation of the alimentary glands, particularly the liver, to an increased output of this enzyme.

2. The decrease in oxidation after the administration of an inorganic acid, such as hydrochloric, to rabbits is attributed to a decrease in catalase brought about by the inhibiting effect of the acid and by the

direct destruction of the enzyme. The increase in oxidation following the ingestion of organic acids, such as acetic, proprionic and butyric, is attributed to an increase in catalase.

3. The increase in oxidation after the ingestion of the amino acids is attributed to the stimulation of the liver to an increased output of catalase by the ammonium carbonate and the organic acids resulting from the deamination of the amino acids, as well as by the amino acids themselves. The increase in catalase after the introduction of hydrochloric acid into the intestine of the dog is attributed to the stimulation of the liver to an increased output of this enzyme by the ammonium chloride formed in the neutralization of this acid by ammonia.

4. The increased oxidation in the sprouting of potatoes and the germination of grain with resulting increase in metabolism and development is attributed to an increase in catalase.

5. It is suggested that the low respiratory metabolism of the ovum before fertilization may be attributed to the low catalase content of the egg, while the increase in the respiratory metabolism after fertilization with resulting development may be due to an increase in catalase brought about by the stimulation of the egg by the spermatazoön to an increased production of catalase. Similarly the relatively low metabolism of the newly-born may be attributed to the poorness of the tissues in catalase, due to the small output of this enzyme from the liver, while the high metabolism characteristic of childhood and youth is a result of the richness of the tissues in catalase brought about by a large output of this enzyme from the liver.

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ON THE PERMEABILITY OF THE PLACENTA FOR ADRENALIN IN THE PREGNANT RABBIT AND ALBINO RAT[1]

YOSHITAKA SHIMIDZU

From the Wistar Institute of Anatomy and Biology, Philadelphia

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INTRODUCTION

At the present time the hormones are of great interest to many investigators. Not only the specific character of each hormone, but the interrelations between them give rise to very interesting problems and are the subject of many discussions. Under normal conditions the interrelation of the several hormones is self-regulated so as to maintain the physiological balance and keep the animal normal. Whenever this equilibrium is disturbed, there occurs a compensatory formation of hormones in several endocrine glands which tends to bring about a readjustment. Sometimes in consequence of this forced activity histological changes in these glands are more or less demonstrable.

During pregnancy the corpus luteum elaborates its hormone actively and consequently calls into responsive action, it is said, some of the other endocrine glands, as the thyroid, hypophysis, suprarenals, etc. Meanwhile, the ductless glands of the fetus develop to a certain degree and begin their function as endocrine organs and their products, including the hormones of the sex glands, are present in the fetal circulation. In multiparous mammals there may be several fetuses of both sexes in one uterus. Thus one pregnant animal incloses several hormone systems in her body, but in deciduate mammals these hormone systems are separated in a measure from each other by the placenta.

From such considerations the important question inevitably arises whether the placenta is permeable for the hormones or not. If the placenta is permeable, there will be a constant exchange of hormones

[1] The observations on the rabbits were made at the Zoölogical Laboratory of the University of Chicago. I wish to acknowledge my obligation to Prof. F. R. Lillie who gave me the best of laboratory facilities and gave me also many helpful suggestions.

not only between mother and fetus, but also between the fetuses themselves, though indirectly, by the intermediation of the mother's circulation, so that the male hormone, for example, will be delivered to the mother and the female fetus, and the hormone of the mother and the female fetus to the male fetus. Thus the interrelation of these hormones or hormone systems becomes much more complicated in pregnancy than in the non-pregnant state. From this point of view, the problem of the permeability of the placenta is very important, not only for the development of the fetus but also for the differentiation of sex.

For the solution of this question it seems to be important as a first step to learn whether a hormone administered to a pregnant animal can be transferred to the fetus through the placenta. As is well known, most of the hormones have not yet been isolated in a pure state; consequently their chemical character and constitution are still obscure, except in the case of adrenalin. Of course adrenalin may not represent the entire suprarenal hormone, but there is no doubt that adrenalin is an essential component of the suprarenal hormone, and we are justified therefore in taking adrenalin as a representative of the suprarenal hormone. If, in the first instance, we could get an experimental result by using such a well-characterized substance, we might apply the result to the next experiment and so gradually approach the solution of the problem.

With this idea in mind I have examined the effect on the fetus of the injection of adrenalin into a pregnant animal, using for this purpose rabbits and albino rats.

GENERAL PLAN OF EXPERIMENTS

The aim of my present experiment is simply to learn whether adrenalin administered to a pregnant animal is able to increase the adrenalin in the contained fetus.

At the end of pregnancy the suprarenal glands of the fetus elaborate their internal secretion and the fetal blood contains a certain amount of adrenalin. Therefore the proof of the presence of adrenalin in the blood of the fetus does not give a conclusive answer to the question. In order to say that the adrenalin administered to the mother has passed to the fetus, there must be a significant increase of adrenalin in the fetal blood as compared with the amount normally there.

There are several chemical and biological methods used to test the presence of adrenalin. In testing for adrenalin in blood the biological

method is considered in general to be better than the chemical. Among the biological methods that which depends on changes in blood pressure seems to be the most sensitive. But, because of its small size, it is almost impossible to measure the blood pressure of a fetus or a newborn individual of the ordinary laboratory animals. A considerable amount of blood is also required in order to examine the effect of adrenalin on the response of smooth muscle. Trendelenburg's method (1) also needs too much blood for my case. The frog-iris reaction requires only a few drops of blood and the manipulation is very simple, but the result is not always reliable.

It is a well-known fact that the parenteral administration of adrenalin causes hyperglycemia, and the animal is generally pretty sensitive to this test. If any increase of adrenalin occurs, it will be followed by hyperglycemia, without fail.

Thus, we can diagnose readily any increase of adrenalin by a quantitative determination of the sugar in the blood, and if we employ a micro-method, the blood sugar can be determined with a little blood, not exceeding the quantity that can be obtained easily from a fetus or several fetuses. Relying on this reaction, I injected adrenalin into pregnant animals and determined the blood sugar in the contained fetuses. Of course, the increase in sugar is not exactly proportional to the increase in the quantity of adrenalin, but I do not require that in my case because in my experiment it is necessary only to determine whether or not the adrenalin in the fetal blood has increased after the injection of adrenalin into the mother. Furthermore, it should be mentioned here that glucose, as is well known, very easily passes through the placenta and thus sugar in the mother's blood, increased by adrenalin, may increase the sugar in the blood of the fetus and thus obscure the real adrenalin effect on the fetus. To avoid such a result special precautions were taken, and these will be discussed later on.

For experimental material, as already mentioned, I used rabbits and albino rats, and made parallel experiments on both these species.

In my experiments the adrenalin used was that prepared by Parke, Davis & Co. The adrenalin was diluted with physiological salt solution before injection and the hypodermic injection was always made between the scapulae.

I. Experiments on rabbits

As the rabbit is the most convenient and the most thoroughly studied animal in respect of adrenalin hyperglycemia, I took this animal first for my tests.

For the estimation of blood sugar in the rabbit I employed the Lewis-Benedict method (2) following the modification of Pearce (3). This method originally requires 2 cc. of blood for one determination, but it is almost impossible to obtain such an amount of blood from one rabbit fetus. Therefore I cut down the quantity of blood for one determination to one-fourth this amount, keeping all the other conditions—picric acid concentration, sodium carbonate concentration, etc., exactly as in the original formula. The sugar determination was made as follows:

To 2 cc. of water 0.5 cc. of blood was added. After the laking of the blood had taken place, 3.75 cc. of a saturated picric acid solution were added, and the solution agitated. To 3 cc. of the filtrate 1 cc. of saturated picric acid solution and 0.5 cc. of 10 per cent sodium carbonate solution were added. The mixture was heated in the autoclave for thirty minutes under a pressure of 20 pounds per square centimeter. Then the solution was made up to 5 cc. with distilled water and put into the colorimeter tube.

The Duboscq colorimeter is too big for my purpose and the Duboscq micro-colorimeter was not at hand, so I constructed a simple micro-colorimeter, using the parts of a burette and some other ordinary laboratory apparatus, and thus imitated the Duboscq instrument. This homemade micro-colorimeter gave me satisfactory results. Before proceeding to the experiment proper, I made some necessary preliminary tests.

Adrenalin hyperglycemia in normal rabbits. Concerning the increase in the blood sugar in rabbits after the adrenalin injection, there are many reports in the literature. I wished to determine this relation using the adrenalin which had been selected and the modified procedure of the Lewis-Benedict-Pearce method arranged for my own experiments.

Three rabbits were used for this purpose. During the night previous to the experiment the animals were kept without food. The next morning each animal received one subcutaneous injection of 5 cc. of 1:10,000 adrenalin. Just before the injection and after it at intervals of one-half, one, two, three and four hours the blood was taken from the ear vein of each animal. The quantity of sugar found in each specimen of blood is given in table 1. On the whole this result agrees with those previously reported.

Adrenalin hyperglycemia in newborn rabbits. There is no doubt that adrenalin causes hyperglycemia in adult rabbits. Now what influence will the adrenalin injection have on the blood sugar in newborn rabbits?

Though the cause of the adrenalin hyperglycemia is not yet clearly explained, it is generally considered as due to the mobilization of gly-

cogen deposited in the body tissues. Therefore if the newborn rabbit has any glycogen deposit in its body, hyperglycemia should be caused by adrenalin. Chipman (4) examined the glycogen of the fetal liver of the rabbit embryo and found that the glycogen appeared at the twenty-second day of gestation and increased rapidly and steadily in amount till the end of pregnancy. Lochhead and Cramer (5) made a quantitative determination of the glycogen in the fetal liver and the remainder of the fetal body in an age series of pregnant rabbits from the fourteenth day to the end of pregnancy.

According to them the fetal liver contains a trace of glycogen at the eighteenth day, though none can be demonstrated histologically till the twenty-second day. At the twenty-fifth day the percentage of glycogen in the liver rises for the first time above the glycogen percentage in the rest of the fetal body, and then there is a rapid increase till on the

TABLE 1

Adrenalin hyperglycemia in rabbits (0.5 mgm. of adrenalin administered subcutaneously)

NUMBER	BODY WEIGHT	BEFORE INJECTION	PERCENTAGE OF BLOOD SUGAR HOURS AFTER INJECTION				
			½	1	2	3	4
	grams						
1	2360	0.08	0.12	0.26	0.26	0.23	0.16
2	2082	0.07	0.18	0.28	0.26	0.23	0.20
3	2187	0.10	0.20	0.31	0.28	0.25	0.21
Average		0.08	0.17	0.28	0.27	0.24	0.19

twenty-ninth day half of the fetal glycogen is stored in the liver. From these facts we can expect the sugar increase in the blood of newborn rabbits after adrenalin injection just as it occurs in the case of adult rabbits. With this expectation I tried the following experiments:

Rabbit 1. At full term of pregnancy;[2] body weight 2925 grams. Four fetuses were excised by Caesarean section. The body weight of the young varied from 34 to 38 grams. Immediately after removal one fetus was killed by bleeding from

[2] In my experiment the copulation of the rabbits was not watched, because it was not so necessary in my case to know the exact age of the fetus. My experiment required only rabbits in advanced pregnancy whose fetuses would have plenty of glycogen in their bodies and, at the same time, blood enough for a sugar determination. In rabbits the full term of pregnancy can be diagnosed without any difficulty. Several hours before the parturition they always begin to make a nest. This manoeuver is very evident and no one can miss it.

the neck while each of the others received 0.1 cc. of 1:10,000 adrenalin. At intervals varying from thirty minutes to two hours after the injection these were killed one after the other for blood specimens. The results of the sugar determinations are given in table 2.

Rabbit 2. At full term of pregnancy; body weight 2737 grams. Five fetuses were obtained by Caesarean section. The body weight of the young varied from 35 to 40 grams. The young were treated in the same way as those of rabbit 1. The percentages of blood sugar at different times are entered parallel with those for no. 1 in table 2.

This result shows that after the adrenalin injection the blood sugar increases in newborn rabbits as in the adults.

TABLE 2

Blood sugar of newborn rabbits before and after the adrenalin injection (0.01 mgm. adrenalin subcutaneously)

NUMBER	PERCENTAGE OF BLOOD SUGAR				
	Before injection	Hours after injection			
		½	1	1½	2
1	0.06	0.14	0.22		0.16
2	0.08	0.12	0.20	0.21	0.18

The influence of adrenalin injected into a pregnant rabbit on the blood sugar in the contained fetus. From the results of the preliminary experiments described above, we can expect an increase of sugar in the fetal blood after the injection of adrenalin into the mother, if the injected adrenalin is transferred to the fetus. As already mentioned, however, the increased sugar in the mother's blood is easily diffusible into the fetus. The increase of sugar in the fetal blood produced in this way is not only meaningless from the standpoint of my experiment, but tends to confuse any possible effect due to the passing of adrenalin into the fetus. Fortunately there is a certain space of time between the adrenalin injection and the distinct appearance of hyperglycemia. Therefore, if the fetus is removed from the mother before it is influenced in a marked way by the sugar in the mother's blood, such confusion will be practically avoided.

With these relations in mind, I made the following experiments.

Nine pregnant rabbits at full term were used. Their body weights varied from 2532 to 3112 grams. After a short period of inanition, from four to six hours, the animals each received one hypodermic injection of 5 cc. of 1: 10,000 adrenalin. Then at varying intervals of

ten, twenty and thirty minutes after the injection the fetuses were removed by Caesarean section under ether. Immediately after removal from the mother, one fetus from each litter was killed by bleeding from the neck, while the others were kept warm till they were examined one after another for blood sugar at periods varying from twenty minutes

TABLE 3

Blood sugar in newborn rabbits whose mother received 0.5 mgm. adrenalin before operation for delivery

NUMBER	PERCENTAGE OF BLOOD SUGAR					
	Mother		New born			
	Before injection	At operation	Just after delivery	20 minutes after delivery	40 minutes after delivery	1 hour after delivery
I. Fetuses removed from mother ten minutes after the adrenalin injection						
1	0.10	0.10	0.10	0.09		0.08
2	0.06	0.07	0.06	0.07	0.06	0.05
3	0.09	0.11	0.08	0.08	0.06	
Average.....	0.08	0.09	0.08	0.08	0.06	0.07
II. Fetuses removed from mother 20 minutes after the adrenalin injection						
1	0.07	0.10	0.08	0.08	0.07	0.07
2	0.09	0.13	0.10	0.09		0.08
3	0.06	0.15	0.12	0.10		0.06
Average.....	0.07	0.13	0.10	0.09	0.07	0.07
III. Fetuses removed from mother 30 minutes after adrenalin injection						
1	0.09	0.15	0.12			0.06*
2	0.09	0.14	0.12		0.10	0.09†
3	0.06	0.18	0.15	0.12	0.12	
Average.....	0.08	0.16	0.13	0.12	0.11	0.08
IV. Control, without adrenalin injection						
1		0.09	0.08	0.08	0.07	0.07
2		0.08	0.08	0.08	0.07	0.07
Average.....		0.09	0.08	0.08	0.07	0.07

* Two hours after the delivery the blood sugar = 0.07 per cent

† Two hours after the delivery the blood sugar = 0.07 per cent; three hours after the delivery = 0.05 per cent

to one or more hours after their delivery. Every blood specimen was used for a sugar determination.

Besides these, two pregnant rabbits at full term were used as control animals. Their body weights were 2435 and 2618 grams respectively. They were treated just as the test animals, but without any injection.

In all cases, in the tests as well as in controls, every placenta was carefully sliced and thoroughly examined for hemorrhages or other abnormalities. Where any pathological change appeared, the animal was omitted from the record. The results of the experiments are given in table 3—I, II, III and IV.

The data in table 3 (I to III) show a difference in the quantity of sugar in the fetal blood immediately after delivery which increases as the interval between the injection and the operation for delivery increases. Nevertheless, the blood sugar of newborn rabbits shows a tendency to decrease in the course of the first hours of extra-uterine life. The interpretation of these facts will be made later.

II. Experiments on albino rats

As the next step in my work I repeated this series of experiments on albino rats. I was fortunately allowed to use pregnant and non-pregnant albino rats from the colony of these animals maintained by the Wistar Institute at Philadelphia.

In consequence of the smallness of this animal I employed the Epstein (6) micro-method for the blood sugar determination. This method requires only 0.1 to 0.2 cc. of blood for one determination and is based on the same principle as the Lewis-Benedict method, which is recognized as an admirable method and was employed by me for rabbits in the present study. The Epstein method does not give very accurate results. However, if it is carefully carried out, it is still fairly available for the usual biological tests, such as this present one, in which accuracy above a certain limit is not necessarily required. The matters to be attended to in connection with this method are the accuracy of the standard color solution and the purity of reagents, especially of the picric acid. Keeping these matters in mind, I got satisfactory results with this method.

In my experiments the rats were brought from the colony house in the morning and kept in the laboratory till the evening, without food, to avoid if possible the alimentary hyperglycemia. Then they received the experimental treatment.

In adult rats the blood was taken from the tail. The fetal blood was obtained from the neck, using two or three fetuses at one time. For one sugar determination 0.1 cc. of blood was always used. The colorimetric work was done almost always under artificial daylight, recommended by Gage (7).[3]

The influence of fear and of ether narcoses on the blood sugar. The rat, even when tamed, is not so gentle as the rabbit and is easily excited by unskilful treatment. Even a hypodermic injection or a sharp cut in the tail sometimes excites the animal greatly. Such an emotional disturbance affects the equilibrium of the blood sugar. Also the ether narcosis, when severe, has the same influence on the blood sugar. In working with such an excitable animal it is absolutely necessary to know first, how much influence these factors have on the blood sugar, because either agitation or narcosis was unavoidable in making the injection and obtaining the blood. To solve this question, I made the following tests with two rats: rat 1, male, body weight 182.4 grams, and rat 2, female, body weight 165.8 grams.

Both of these were roughly treated with the forceps, making them very angry. Each was then wrapped up in a towel and the blood taken by cutting the tail. To compare with these results samples of blood were taken from rat 3, male, body weight 174.6 grams; rat 4, male, body weight 126.9 grams; and rat 5, female, body weight 132.4 grams.

These animals had been treated very gently and carefully; wrapped in a towel, placed on the table softly, without causing any marked excitement, and the blood obtained by cutting the tail.

Finally rat 6, female, body weight 135.3 grams; rat 7, female, body weight 143.6 grams; rat 8, male, body weight 164.7 grams; rat 9, male, body weight 147.8 grams, were lightly etherized and the blood was taken in the same manner from the tail.

The blood sugar determination for each animal gave the results entered in table 4.

[3] The growth of the fetus of the albino rat becomes rapid toward the end of pregnancy. Parturition occurs usually at night. I made my tests therefore almost always at night in order to get the fully-grown fetus, and to obtain as much blood as possible. Thus I was obliged to make the color tests under artificial daylight, because the glucose in the solution diminishes in its reducing power with time and the color of the produced picramate fades pretty rapidly. In my experience the artificial daylight is rather better than natural daylight, if the background is not homogeneous, as is usually the case in cities or towns, where buildings are crowded around the laboratory.

This result shows that the effect of agitation is distinct, while the ether narcosis has no marked influence on the blood sugar, when, as in my case, the narcosis is neither deep nor long.

TABLE 4

Blood sugar in excited, not excited and lightly etherized rats

RAT NUMBER	SEX	BODY WEIGHT	MENTAL CONDITION	ETHER NARCOSIS	BLOOD SUGAR PER CENT	AVERAGE PER CENT
1	♂	182.4	Agitated	−	0.22	0.21
2	♀	165.8	Agitated	−	0.20	
3	♂	174.6	Quiet	−	0.16	0.16
4	♂	126.9	Quiet	−	0.18	
5	♀	132.4	Quiet	−	0.15	
6	♀	135.3	Quiet	+	0.15	0.16
7	♀	143.6	Quiet	+	0.15	
8	♂	164.7	Quiet	+	0.17	
9	♂	147.8	Quiet	+	0.16	

Rats 6 to 9 were narcotized once more at intervals varying from thirty minutes to one hour after the first narcosis. The blood of each animal at that period gave a percentage of sugar as shown in table 5.

TABLE 5

Blood sugar of twice narcotized rats

RAT NUMBER	PERCENTAGE OF BLOOD SUGAR		INTERVAL BETWEEN TWO NARCOSES
	First blood	Second blood	
			minutes
6	0.15	0.15	30
7	0.15	0.14	60
8	0.17	0.16	30
9	0.16	0.16	40

As this table shows, the repeated ether narcosis has no apparent effect on the blood sugar of albino rats, provided the narcosis is neither deep nor long. So we can use ether narcosis without fear of complicating our results.

Adrenalin hyperglycemia in normal albino rats. In the following tests each animal received 0.5 cc. of 1: 10,000 adrenalin subcutaneously. Just before the injection and at intervals varying from thirty minutes to two hours after the injection, the blood was taken from the tail

under light ether narcosis. In the control animals 0.5 cc. of physiological salt solution was injected in the place of the adrenalin. The result of the sugar determinations is given in table 6.

TABLE 6

Adrenalin hyperglycemia in albino rats (0.05 mgm. adrenalin injected subcutaneously)

RAT NUMBER	SEX	BODY WEIGHT	PERCENTAGE OF BLOOD SUGAR				
			Before injection	Hours after injection			
				½	1	1½	2
Controls							
		grams					
1	♂	148.7	0.15	0.16			
2	♂	151.6	0.16	0.16			
3	♀	159.1	0.17		0.17		
4	♀	141.8	0.16		0.16		
5	♂	123.6	0.16			0.18	
6	♂	170.1	0.17			0.15	
7	♂	177.3	0.16				0.16
8	♀	132.2	0.18				0.16
Average			0.17	0.16	0.17	0.17	0.16
Tests							
1	♂	130.4	0.13	0.18			
2	♂	165.5	0.16	0.19			
3	♂	136.8	0.14	0.18			
4	♀	143.2	0.16	0.19			
5	♂	155.6	0.15	0.18			
6	♀	120.8	0.15		0.20		
7	♀	139.3	0.15		0.22		
8	♂	148.7	0.19		0.21		
9	♂	177.1	0.19		0.31		
10	♂	158.1	0.19			0.31	
11	♀	114.3	0.15			0.23	
12	♂	139.2	0.14			0.20	
13	♂	121.8	0.15			0.24	
14	♀	146.3	0.15				0.21
15	♂	167.2	0.17				0.21
16	♂	186.1	0.15				0.19
17	♀	131.5	0.16				0.20
Average			0.16	0.18	0.24	0.25	0.20

Table 6 shows that in albino rats the blood sugar rises slowly after the adrenalin injection, reaching its maximum after one or one and a half hours. Though the percentage of the blood sugar at the highest point of the hyperglycemia is not much less in rats than in rabbits, the increase of the blood sugar is not so apparent as in rabbits, owing to the high percentage of normal blood sugar in the rats. In spite of that difference the albino rat is still a fairly available animal for my purpose.

Adrenalin hyperglycemia in newborn albino rats. The fetuses of three pregnant rats were removed by Caesarean section at full term.[4]

Immediately after delivery by operation two or three fetuses from each litter were used for the first blood specimen, while each of the others received a hypodermic injection of 0.05 cc. of 1 : 50,000 adrenalin, and then were kept in an incubator till they were killed at varying intervals of from thirty minutes to one and a half hours after the injection.

The blood sugar determination gave the results shown in table 7.

TABLE 7

Blood sugar of newborn albino rats before and after the adrenalin injection (0.001 mgm. adrenalin administered subcutaneously)

RAT NUMBER	PERCENTAGE OF BLOOD SUGAR			
	Before injection	Hours after injection		
		½	1	1½
1	0.14	0.18	0.20	
2	0.14	0.17		0.21
3	0.15		0.20	0.20
Average.....	0.14	0.18	0.20	0.21

These results indicate that the newborn albino rats also show a distinct reaction to adrenalin by an increase of the blood sugar. Therefore we can reasonably expect an increase of blood sugar in a fetus after the injection of adrenalin into the mother, if the injected adrenalin is transferred to the fetus, and this sugar increase should continue for one or more hours after the injection, regardless of the removal of the fetus from the mother.

[4] The full term of pregnancy of the rat can be diagnosed by palpation. The growth of the rat fetus in the last days of intra-uterine life is very rapid. The difference between the size of the fetus on the last day and on the day before is quite distinct. Therefore diagnosis of the last day of pregnancy is not very difficult after a little practice.

The influence of the adrenalin injected into pregnant albino rats on the blood sugar of the fetuses. For this series of experiments twelve pregnant rats at full term were used, nine as tests and three controls. Their

TABLE 8

Blood sugar of newborn rats whose mother received 0.05 mgm. adrenalin before operative delivery

NUMBER	PERCENTAGE OF BLOOD SUGAR					
	Mother		New born			
	Before injection	At operation	Just after delivery	20 minutes after delivery	40 minutes after delivery	1 hour after delivery
I. Fetuses removed from mother 10 minutes after injection						
1	0.13	0.13	0.13	0.12	0.12	
2	0.14	0.14	0.15	0.13		0.11
3	0.14	0.14	0.14		0.11	0.12
Average.....	0.14	0.14	0.14	0.13	0.12	0.12
II. Fetuses removed from mother 20 minutes after injection						
1	0.16	0.20	0.16	0.15		0.13
2	0.15	0.18	0.16	0.15	0.13	
3	0.15	0.17	0.16		0.12	0.13
Average.....	0.15	0.18	0.16	0.15	0.13	0.13
III. Fetuses removed from mother 30 minutes after injection						
1	0.17	0.20	0.18		0.14	0.15
2	0.14	0.19	0.17	0.16	0.13	
3	0.15	0.21	0.17	0.15		0.12
Average.....	0.15	0.20	0.17	0.16	0 14	0.14
IV. Controls: without adrenalin injection						
1		0.12	0.12	0.11		0.09
2		0.14	0.14		0.13	0.13
3		0.15	0.14	0.14	0.13	
Average.....		0.14	0.13	0.13	0.13	0.11

body weights varied from 153.6 to 215 grams. Each test animal received one hypodermic injection of 0.5 cc. of 1: 10,000 adrenalin. At an interval of ten, twenty or thirty minutes after the injection, all the

fetuses were removed from each of the group of three animals under light ether narcosis. Just before the injection and the operation respectively, the maternal blood was taken from the tail for the determination of sugar.

Concerning the fetuses: two or three fetuses from each litter were killed for the first fetal blood, immediately after delivery, while the others were kept in an incubator till killed for further blood specimens, at intervals of twenty, forty and sixty minutes after delivery.

The control animals received no injection. Otherwise they were treated in the same way as the test animals. The results of sugar determination for each blood specimen are given in table 8—I, II, III and IV.

If we compare these results on albino rats with those on rabbits, we find a striking similarity between them. Just after delivery the blood sugar of the fetuses is highest in those which remained longest in the mother's body after the adrenalin injection, and vice versa. In all cases, however, the fetal blood sugar decreases to normal or even to a subnormal value in the course of the first hour of extra-uterine life.

GENERAL DISCUSSION

My preliminary experiments show that in both the rabbit and the albino rat the fetuses after removal from the mother distinctly respond to adrenalin as do the adults by an increase in their blood sugar. Therefore, if the increased adrenalin in the maternal blood is able to raise the adrenalin concentration in the fetal blood, the adrenalin injection into a pregnant animal will increase the adrenalin in the fetal blood and consequently increase the fetal blood sugar.

In my experiments, however, there was no indication of an increase of adrenalin in the fetal blood after the injection of adrenalin into the mother. At the moment of delivery the blood of the fetus, which had been left in the mother's body for twenty minutes or more after the injection, showed a little increase of sugar. This slight hyperglycemia of the fetus might be taken erroneously as an effect of increased adrenalin in the fetal circulation. But such is not the case. If we once compare the percentage of sugar of this first fetal blood with that of the maternal blood at the moment of removal of the fetuses, we shall find a striking parallelism between them. Where the content is high in the mother it is also high in the fetus, though always a little lower absolutely than in the mother. Furthermore, we find that the blood

sugar of the fetus, in all cases, gradually decreases in the course of the first hours of its extra-uterine life, both in the tests and in the controls.

This is in contradiction to the course of the hyperglycemia of the fetus, which received an adrenalin injection immediately after delivery. The hyperglycemia caused by addition of adrenalin continued to increase for at least one hour after the animal received adrenalin. Therefore, if this hyperglycemia of the fetus were really caused by an increase of adrenalin in the fetal circulation, the blood sugar should continue its increase even after birth, because the fetus was removed from the mother at least within the first half of the supposed period of increment.

From such considerations we naturally come to the conclusion that adrenalin cannot be increased in the fetal circulation by injection of it into the mother; in other words, adrenalin injected into the pregnant animal does not pass through the placenta to the fetus. The high content of sugar in the fetal blood at the moment of birth is to be explained as a result of the diffusion of the increased sugar in the maternal blood toward the fetus.

To explain this negative result we must consider first the character of adrenalin, which is said to be easily destroyed in the living tissue under certain conditions. Numerous investigations regarding the fate of injected adrenalin in the animal body have been reported. In most of these investigations adrenalin was injected directly into the circulation and the effect of the drug on the blood pressure or on the tonus of the blood vessel was used as an indicator of its presence. The rise of the blood pressure after the intravenous injection of the drug lasts only a short time—thirty seconds to two to three minutes, according to the dose applied. Though this rapid cessation of the action of adrenalin on the blood pressure seems to be in favor of rapid destruction of the drug, we are far from having data that would justify us in supposing that the fall of blood pressure indicates the disappearance of adrenalin from the circulation.

Weiss and Harris (8) injected adrenalin and observed the circulation in the swimming membranes of the frog on both sides, while the arteria iliaca on one side was compressed. After the contraction of vessels on one side had completely relaxed, the vessels on the other side began to contract, when the ligature was released. In cats the blood, which no longer gave the adrenalin effect on the vessels, was however able to raise the blood pressure in the other animal, when it was injected. Again Miller (9) affirmed that the active substance does not disappear from the blood, and the blood of a rabbit, which has received a dose

of adrenalin, will cause a typical rise of blood pressure if injected into another rabbit, although the effect upon the first animal may have disappeared. Kahn (10) detected adrenalin in the blood by means of Trendelenburg's frog vessel preparation after the complete disappearance of adrenalin effect on the blood pressure of the injected animal, when the dose used was not too small.

Though there are many contradictory reports, these investigations just cited justify us in assuming that the intravenously injected adrenalin remains in the circulation even after the cessation of its effect on the blood pressure.

Moreover, many drugs, when subcutaneously injected, behave quite differently from those intravenously injected. The absorption is generally slower by subcutaneous injection than by intravenous injection. This is especially the case for adrenalin. The absorption of subcutaneously injected adrenalin is distinctly slowed by the contraction of the vessels around the point of injection. While the intravenous injection of adrenalin distinctly raises the blood pressure, the subcutaneous injection, at least in animals, does not raise the blood pressure, even in a large dose. After subcutaneous injection, therefore, we cannot use the effect of adrenalin on the blood pressure as an indicator of the drug. We have used here the effect of adrenalin on the sugar metabolism as an indicator, in the place of blood pressure.

Regarding the proportion of the quantity preserved to that destroyed when injection is made under the skin, we are unable to make a definite statement. Ritzmann (11) reports that 2 mgm. of adrenalin subcutaneously injected are equal to 0.4 mgm. intravenously injected, when measured by the amount of sugar appearing in the urine. From this fact we may suppose that about four-fifths of the subcutaneously injected adrenalin may be destroyed under the skin, before it enters the circulation. Even though the greater part of the injected adrenalin may undergo a destruction under the skin, it is improbable that this destruction occurs at the moment of injection, otherwise the absorption of one-fifth of the dose can be hardly explained. In reality this destruction does not seem to be so rapid. Embden and Fürth (12) report that 0.1 gram of suprarenin chloride disappears almost completely in two hours if added to 200 cc. of defibrinated beef blood, and if the mixture is constantly aërated at body temperature.

Furthermore, it is a wellknown fact that in man a subcutaneous injection of 0.5 mgm. adrenalin raises the blood pressure after 5 to 12 minutes. The maximal height continues for 2 or 3 hours and is then followed by a gradual fall.

Therefore it is reasonable to assume that by subcutaneous injection the drug is absorbed gradually and slowly by the circulation, and consequently the adrenalinemia will last a considerably long time, at least longer than that caused by an intravenous injection.

As stated already, I injected adrenalin hypodermically (0.5 mgm. for rabbit, 0.05 mgm. for albino rat) and examined the blood sugar of the fetus at intervals varying from ten to thirty minutes after the injection, but I found no indication of an increase of adrenalin in the fetal blood. In giving an explanation of this negative result, three factors are to be considered: first, the quantity of adrenalin administered; second, the duration of adrenalinemia of the mother animal; and third, the impermeability of the placenta for adrenalin.

Concerning the first factor there can be no doubt that the quantity administered was sufficient for my purpose, even on the assumption that nine-tenths or more of the injected drug is destroyed under the skin. Moreover, my experiment itself clearly proves that the quantity was sufficient, because every injection caused a strong hyperglycemia in the mother animal.

To answer the question as to how long the adrenalinemia of the mother animal lasted, is not easy. However, as mentioned above, we have reason to believe that the adrenalinemia caused by a subcutaneous injection of adrenalin lasts for a long time. In my experiments thirty minutes was the longest interval from the injection to the removal of the fetus. During this interval, the increased blood sugar of the mother had already passed to the fetus in a considerable amount, but no trace of adrenalinemia was to be seen in the fetus, despite the length of this interval, which ought to have been long enough for the passage of such a crystalline substance through the placenta.

It appears therefore that the quantity of adrenalin was enough and the interval for the transfer of adrenalin from mother to fetus was also sufficient. Consequently if adrenalin were able to pass the placenta, there must have been an adrenalinemia also in the fetus, but the results are absolutely negative. We find the explanation of this negative result in the third factor, the impermeability of the placenta for adrenalin. On the basis of these few experiments, of course, I do not dare to say that the placenta is absolutely impermeable for adrenalin, but it seems certain, at least, that we cannot increase adrenalin in the fetal blood by a subcutaneous injection of the drug into the mother animal. Whether or not a more severe and longer continued adrenalinemia of the pregnant animal can increase the adrenalin in the fetal blood, is still an open question.

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THE CHARACTER OF THE SYMPATHETIC INNERVATION TO THE RETRACTOR PENIS MUSCLE OF THE DOG

CHARLES W. EDMUNDS

From the Pharmacological Laboratory of the University of Michigan

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In recent years the retractor penis muscle has attracted considerable attention from anatomists and physiologists as it has served as an object for the study of the physiology of smooth muscle for which work it is especially well suited, on account of the simple arrangement of its fibers. A second reason which has aided in making it useful in physiological and pharmacological experimental work is its innervation which, as described, is simpler than that of most of the smooth muscle structures. As Fröhlich and Loewi (1) point out, some of the more complicated structures as, for example, the vessels and hollow organs possess a fourfold innervation while the simply constructed retractor muscle has only a double innervation.

The muscle was first described by Eckhard, but for our knowledge of its innervation we are indebted to Langley and Anderson (2), who found that the motor fibers for the muscle were contained in the lumbar sympathetics, the fibers running to the muscle chiefly in the nervus dorsalis penis., They state further (loc. cit., p. 93) that they could find no satisfactory evidence of the presence in the lumbar nerves of inhibitory fibers for the external generative organs either in the male or in the female.

The inhibitory fibers they found came from the pelvic nerves being contained in the nervi erigentes. The evidence in favor of the correctness of this statement I have summarized elsewhere (3).

These views of the nerve supply of this muscle as described by Langley and Anderson have been accepted apparently by all subsequent workers upon this structure and the various text-books which refer to it quote the above mentioned work as their authority. Reference may be made to Fletcher (4) and to Schäfer (5); to De Zilwa (6), Fröhlich and Loewi (loc. cit.), and to Brücke (7), and finally to Bottazzi (8), who has carried out a very extensive research upon the physiology of smooth muscle using the retractor as the test object.

Present research. My own experiments with this muscle were carried out in connection with some investigations that were being made upon the pharmacology of the bladder and in an effort to explain the results which were obtained upon that organ. These results are described elsewhere (3).

Most of the work in that research was carried out upon the isolated muscle immersed in oxygenated Ringer's solution in the usual manner and maintained at a temperature of between 34.5°C. and 35°C., which temperature appeared to suit its activity best. Confirmatory experiments were carried out upon the intact dog. Pieces of the retractor muscle prepared in this way usually start up an automatic rhythm in from ten to thirty minutes and these movements will continue over long periods of time if the oxygen supply and the proper temperature are maintained.

If to the Ringer's solution in which the muscle is immersed, a drop or two of a solution of adrenalin chloride (1-1000) is added, the muscle promptly contracts in harmony with the well-known rule that adrenalin results are similar to those produced by sympathetic stimulation.

In connection with my experiments upon pilocarpine and other alkaloids upon the retractor, I found it necessary to paralyze the sympathetic endings in order to eliminate that structure as a possible point of attack for these alkaloids. For this purpose a small amount of ergotoxin was added to the Ringer's solution, and the muscle was allowed to remain exposed to it for a short time. To the muscle in fresh Ringer's solution adrenalin was added as before in order to test the completeness of the paralysis and now in place of the adrenalin producing a contraction or being inactive as was to be expected, the muscle responded by a marked relaxation. The most likely explanation of this phenomenon when taken into consideration with the action of adrenalin after ergotoxin elsewhere in the body was that inhibitory nerves were present in the sympathetic and that these are ordinarily masked by the more powerful motor fibers and are only able to demonstrate an action when the motor fibers are paralyzed. These results obtained with adrenalin after ergotoxin were confirmed upon other preparations of the isolated retractor with results entirely similar to those described above, but in order to prove the correctness of the explanation offered, it was necessary to repeat the experiments using the entire animal instead of the isolated muscle and testing at the same time the sympathetic nerve for a possible alteration in its reaction.

Accordingly a medium sized dog was anaesthetized with chloretone dissolved in oil and after anaesthesia was complete tracheal and venous cannulae were inserted. The abdomen was then opened and the hypogastric nerve was isolated and prepared for stimulation. The retractor muscle was next exposed and the peripheral end cut from its point of insertion and the muscle separated from its fascial covering. The peripheral end of the muscle was connected with a recording lever and the muscle itself protected from drying by a covering of absorbent cotton kept moist with warm salt solution.

FIG. 1

FIG. 2

Fig. 1. Adrenalin upon the normal isolated retractor penis muscle. Lever moves down in contraction.

Fig. 2. Adrenalin upon the isolated retractor muscle which has been subjected to action of ergotoxin. Lever moves down in contraction.

Injections of adrenalin and sympathetic stimulation, obtained as controls, were followed by the customary contractions. Ergotoxin was now injected into the jugular vein and the progress and extent of the paralysis was tested by repeated injections of adrenalin. It was found that the nerves were quite resistant to the action of the ergotoxin, three injections of ergotoxin being necessary before the motor effects of the adrenalin upon the blood pressure were obliterated. When this stage was reached not only did the retractor respond to the adrenalin by relaxation but electrical stimulation of the hypogastric was also followed by an elongation of the muscle thus demonstrating the presence of

inhibitory fibers in the sympathetic nerve to the retractor and bringing this structure more closely into harmony with those abdominal organs possessing a double innervation.

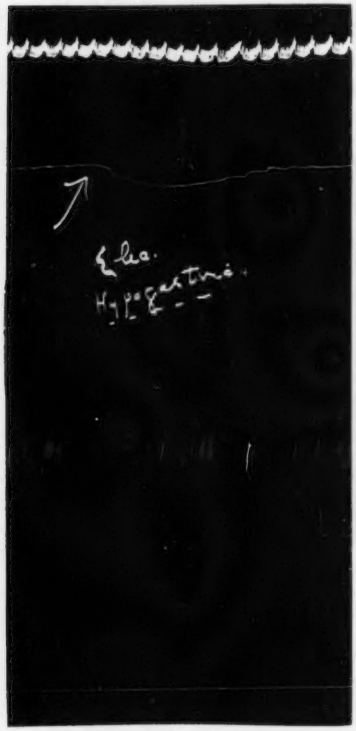

FIG. 3

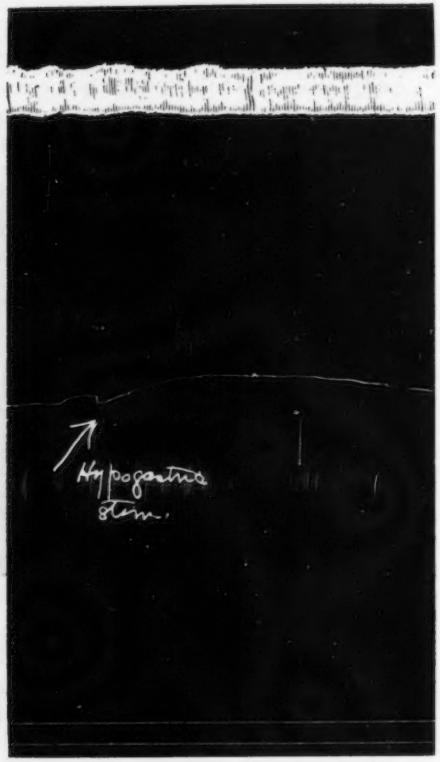

FIG. 4

Fig. 3. Tracing of blood pressure in dog with effect of stimulation of sympathetic nerve upon the retractor muscle. Lever moves down in contraction.

Fig. 4. Blood pressure in same dog as shown in figure 3 with result of hypogastric stimulation on retractor muscle after ergotoxin has paralyzed the motor fibers. Lever moves up as muscle relaxes.

Figures 1 and 2 show the effect of adrenalin upon the isolated retractor muscle before and after ergotoxin, while figures 3 and 4 were obtained from the retractor muscle in an intact dog and show the effect of sympathetic stimulation before and after the use of ergotoxin.

SUMMARY

The sympathetic nerve to the retractor penis muscle of the dog contains inhibitory as well as motor fibers.

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ENERGY EXPENDITURE IN HOUSEHOLD TASKS

C. F. LANGWORTHY AND H. G. BAROTT

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The expenditure of energy by an individual is influenced by a number of factors, among the more significant of which are age, size, sex and occupation. The effect of some of them is relatively constant, while that of others varies decidedly with circumstances. This is especially true with regard to occupation. The expenditure of energy in the performance of any task varies with the nature of the task and with the rate at which it is performed. If the effect which the several factors have had upon the transformations of energy in the body were known and the length of time each was effective, it would be possible to gauge the energy requirements of an individual in the various circumstances of his daily life. Furthermore, information of this sort is essential in estimating the amounts and kinds of food required by an individual to meet the needs of his body for energy.

For a number of years the work of the Office of Home Economics has included investigations of these various factors as affecting both men and women. The present article presents the results of some studies of the energy requirement for the performance of several household tasks. These experiments are part of an extended and comprehensive series which included not only measurements of the energy expenditure in the performance of the different kinds of tasks and of similiar tasks under different conditions, but also the amount of time expended in such tasks under varying conditions of equipment, personnel and routine.

Variations in the transformation of energy in the body are accompanied by corresponding variations in the heat output, and the expenditure of energy by the body is estimated from measurements of the heat produced. Such measurements are made by means of a respiration calorimeter. A series of experiments, which has been referred to in reports of the Department of Agriculture (1), was made with one of

the calorimeters in the laboratory of the Office of Home Economics which was described in a publication of the Department (2). A report of the results of these experiments, which is now being prepared, was interrupted by the entrance of the investigator who conducted them into the military service. The experiments here reported are a continuation of the work, but with the respiration calorimeter somewhat modified to overcome difficulties that had been experienced in the course of the preceding series. The size of the chamber had been reduced to increase the reliability of the measurement of oxygen consumed by the subject, and the mass of metal in the structural framework of the apparatus had been removed because its presence introduced difficulties in securing accurate data regarding the heat conditions of the chamber whenever any change was made in the activities of the subject and consequently in the rate of production of heat within the chamber.

Briefly stated, the respiration calorimeter consists of a chamber (75 x 120 x 200 cm.) with air-tight walls, connected with devices for maintaining a current of air through it from which the water vapor and carbon dioxid eliminated by the subject within the chamber can be removed, and equipped with devices for carrying away and measuring the quantity of heat produced within the chamber. The subject of the experiments remains in this apparatus during the experimental period, resting or performing some specified task in accordance with the plan of the experiment. A comparison of the amount of energy transformed by the body during a given period of time while at complete rest and while doing the prescribed work is taken as an indication of the requirement of energy for the performance of the specified task.

With an apparatus of this sort energy expenditure may be determined in two ways, directly by calorimetric measurements of the heat produced by the body, and indirectly by computation from the amount of oxygen consumed and carbon dioxid produced by the subject. In the present experiments the two methods gave results that were practically identical. Only the figures obtained by the direct measurement of heat are here discussed as these are fundamentally the more accurate.

PLAN OF EXPERIMENTS

The subject for this series of experiments was a woman twenty-two years old, of rather thin and spare build, 5 feet 4 inches tall, and weighing 110 pounds (50 K) in uniform, her weight being taken at the end

of each experiment. The uniform, consisting of a "middy blouse" and skirt of cotton with very light underclothing, was the same in each experiment. The experimental period, which was always of two hours' duration, started at approximately the same time each day so as to obtain as far as possible the same physiological conditions. The subject ate no breakfast, but about 7:30 each morning she drank one cup of cocoa made uniformly of half a pint of cream, 2 teaspoonsful of cocoa and 1 teaspoonful of sugar. She arrived at the laboratory so as to enter the calorimeter about 10:30 a.m., and measurements of the experimental data started between 11:00 and 11:30. While in the calorimeter she followed a routine that was carefully planned previous to the experiment and performed all her work to the beat of a metronome, so that an exact count of the number of movements was possible. Therefore, a complete time-motion study is provided for each kind of work. The data thus obtained may be used for comparison with the energy required for any similar task actually performed in ordinary life where the rate of work may be either faster or slower. The series includes experiments with the subject at rest and performing various household tasks.

The tasks performed during these preliminary experiments were chosen partly because they represent some of the more common and typical household activities and partly because they seemed likely to indicate how experimental methods should be developed for this type of investigation, especially in the matter of reproducing actual working conditions within the calorimeter. In the experiments with washing no water was used because of the complicating effect it would have on the water vapor and heat measurements, but it is hoped to devise means of overcoming this difficulty in later experiments. The results of fifty-three tests (briefly noted in the report of the work of the Office of Home Economics, presented at the 11th annual meeting of the American Home Economics Association, Science Section, Chicago, June, 1918) (3), are summarized in table 1.

Rest experiments. The first measurements of energy expenditure by this subject were made in four rest experiments in which the external muscular activity was reduced to a minimum. For this purpose the subject rested easily in a comfortable position in a large swivel chair, and remained very quiet, hardly moving during the two hours comprising each rest experiment. The number of calories measured during each of the four experiments was very uniform, indicating that the expenditure of energy by this subject while sitting quietly was practically

constant. The average of the values for these four experiments is used as a basis of comparison with those for experiments in which work was performed, showing the increase in heat output resulting from the muscular activity involved in the performance of the work.

Experiments with knitting, crocheting and hand sewing. The measurements of the energy expended while knitting, crocheting and hand

TABLE 1

Average energy (heat) output during experiments with a woman engaged in different household tasks

CHARACTER OF EXPERIMENT	NUMBER OF EXPERIMENTS	HEAT ELIMINATION			
		Total per 2-hour period	Total per hour	Per hour per kilogram of body weight	Per hour for work alone
		calories	*calories*	*calories*	*calories*
Resting	4	121.4	60.7	1.22	
Knitting	4	141.2	70.6	1.42	10.1
Crocheting	5	138.0	69.0	1.39	8.3
Sewing					
Scalloping	2	140.8	70.4	1.43	10.2
Running	2	136.4	68.2	1.39	7.5
Hemming	2	139.6	69.8	1.45	10.3
Hemming and running	3	142.4	71.2	1.39	9.8
Darning	2	137.4	68.7	1.40	8.0
Average for sewing	11	139.6	69.8	1.40	9.4
Dressing infant (model)	4	168.8	84.4	1.72	23.6
Sweeping floor	4	201.4	100.7	2.06	40.1
Washing floor	3	177.6	88.8	1.82	29.0
Washing towels	4	220.8	110.4	2.22	49.6
Ironing towels	6	171.6	85.8	1.75	24.3
Dishwashing					
Table 65 cm. high	2	181.6	90.8	1.85	30.0
Table 100 cm. high	4	190.2	85.1	1.74	24.4
Table 85 cm. high	2	162.2	81.1	1.65	20.3
Average for dishwashing	8	181.1	90.5	1.75	24.8

sewing, were all conducted with the subject comfortably seated in an ordinary bentwood chair, and all motions except those necessary for the work were reduced to a minimum.

The knitting was done on a sweater about half completed. The rate of work was 23 stitches per minute taken to the beat of a metronome.

In the crocheting experiments, fine cotton thread was used and a simple pattern chosen. The rate of the work was 32 stitches per minute.

The experiments with hand sewing included several types of work: *a*, making a plain unpadded scallop (blanket stitch) on the edge of a small piece of fine linen, at the rate of 18 stitches per minute; *b*, simple running on light cotton goods, 6 stitches being taken on the needle, one to one beat of the metronome, then the thread pulled through to four beats, with a total of 30 stitches per minute; *c*, hemming on light cotton goods, at a rate of 30 stitches to the minute; the thread being pulled all the way through after each stitch; *d*, darning light-weight cotton hose with a thread about 24 inches long.

As might be expected these tasks all entail a relatively small expenditure of energy, varying from 7 calories per hour for sewing with the running stitch to 10 calories per hour in hemming. The expenditure during knitting (10 to 11 calories per hour) is slightly higher than that during crocheting (8 to 9 calories), doubtless because the wool sweater which was handled in knitting weighed more than the cotton lace which was being crocheted, and because greater play of the hands and arms was required by knitting with fairly long needles than by the crocheting. Since in the sewing experiments the materials used were of approximately the same weight and ease of handling, the differences there are probably to be attributed to the extent of movement required; thus with the running stitch (7 calories per hour) the movement of drawing the thread to its full length was made only every 6 stitches or 5 times per minute, whereas with the hemming (10 to 11 calories per hour) it was made after each stitch or 30 times per minute. This is in line with the practical experience of needleworkers, who for most forms of plain sewing avoid an unnecessarily long thread as causing too much work of the arms. In later experiments it is planned to introduce sewing on articles of different types in order to learn how such factors as weight, shape and texture influence the energy expenditure.

Experiments on dressing model of infant. It was desirable that some observation be made on the energy output involved in caring for children and the task of dressing and undressing an infant was chosen for this preliminary test. Because of the difficulties in conducting the experiment with a young child, a full-sized model was substituted. The model was about the size of a year-old infant, but it weighed, dressed, only 2 kgm., whereas a child of this size would weigh about 8 to 10 kgm. The clothing of the model consisted of a band (no sleeves), diaper, shirt with sleeves, two underskirts (no sleeves), dress, knitted

sack with sleeves, socks, booties and bonnet. The model was dressed and undressed seven times during each 2-hour experiment.

The energy output in this experiment, 23.6 calories per hour for work alone, is undoubtedly lower than would be required in the care of a living child, as a child of this size would be four or five times as heavy to lift and its movements would still further increase the energy output. These figures, therefore, represent only the labor involved in the act of removing and replacing the clothes plus the necessary lifting about and supporting of a 2-kilogram model.

Even the unduly low figure thus obtained (24 calories in round numbers per hour) was fully twice as great as that for such light tasks as sewing, similar to that required for dishwashing and ironing and about one-half that for washing clothes. This indicates that such work should be classed as a moderately heavy household task. A part of the program for experiments now being conducted is the measurement of energy expended in the care of living children and it is believed that the value of the results thus obtained will be sufficient to offset the difficulties involved.

Experiments with sweeping and washing floors. These tasks were included as representing what are usually considered rather hard forms of housework. The sweeping was done on the bare floor of the calorimeter. A long-handled broom was pushed forward on the floor, lifted and moved back. The rate was 38 complete strokes per minute.

In the floor-washing experiment, the motions of actual washing were simulated with a dry cloth and empty pail. The subject worked on her knees, giving 85 short rubs of the cloth on the floor in 50 seconds and wringing the cloth in the pail for 10 seconds.

When compared with such tasks as knitting and sewing, sweeping the floor would be classified as hard labor, the energy expenditure, 40.1 calories per hour for work alone, being four or five times as great. Plans have been made for experiments with various types of floor coverings. This work should give interesting results as to the relative resistance offered to the broom by other surfaces as compared with the smooth floor of the calorimeter, and the consequent variations in the energy required for sweeping.

The average of 29 calories per hour for energy expenditure during floor washing is believed to be considerably too low for usual work of this kind. Not only was the task slightly lightened by omitting water, which would add to the work of lifting and wringing the cloth, but the subject, who was unused to such labor, was not able to continue exerting

as much force as a professional charwoman would expend and her movements were those of light rubbing rather than hard scrubbing. It is planned to repeat the experiment with a stronger subject accustomed to such tasks.

Experiments with laundry work. In the experiments with laundry work, both washing and ironing were included. The work was done on towels 16 inches square.

In washing, the equipment consisted of a small galvanized iron tub and an ordinary scrubbing board. A towel was rubbed on the board 40 times in 30 seconds, wrung by hand for 15 seconds, then exchanged for another towel, 15 seconds being allowed for the exchange. This routine was repeated throughout the experiment.

The energy output in washing was 49.6 calories per hour for work alone; this is similar to that found for sweeping floors and like it must be classified as hard labor. It seems probable that the weight of the water in actual washing might increase the labor of moving the towel and also that wet articles might offer slightly more resistance on the scrubbing board and in wringing. Moreover in ordinary family washing the average weight and size of the articles handled are greater than those of the towels used in these tests, and would tend to increase rather than decrease the energy expenditure. The figures here given, therefore, represent less rather than more energy than would be expended for comparable work in actual laundering. It is hoped to devise some way to overcome the difficulties of using water in experiments with such types of work and to approximate more closely the conditions actually found in the household.

In the ironing experiment, the work was done with a 5-pound iron on a table of comfortable working height. The rate of work was 70 strokes of the iron in 50 seconds, and 10 seconds to change towels. A cold iron was used because of the complications which heat would cause in the measurements, but it is not probable that this omission would noticeably affect the amount of energy expended by the worker.

The energy expenditure in the ironing experiments, which in six experiments averaged 24 calories per hour, lies between that of the tasks classed as light work and those classed as heavy work and might be classed as moderate work.

Experiments with dishwashing at tables of different heights. In the dishwashing experiments a table of adjustable height was used so that studies might be made of the comparative amounts of energy expended when working surfaces of different heights were used. In one set of

observations the table was too low for comfort, in another, too high, while in a third it was set at what was regarded as the correct height for the worker.

The dishes, comprising 4 plates, 2 bowls, 2 teacups and 2 saucers, were placed in a pan, rubbed with a cloth, placed in a draining pan and then wiped. The motions were the same as in actual washing. Each dish was given 10 rubs, turned, and given 10 more, in time with a metronome, beating 130 times per minute, then 10 beats were allowed to change pieces. The work simulating drying was then performed at the same rate. The whole process was repeated twelve times per hour.

In the series of tests in which the table top was set too low for the comfort of this subject, the height from the floor to the top of the table was 65 cm. and to the top of the pan 78 cm. In the series in which it was too high, the top of the table was 100 cm. and the top of the dishpan 113 cm. from the floor. In the series in which the height was adjusted to the comfort of the subject the table top was 85 cm. and the top of the pan 98 cm. from the floor.

As regards the general question of energy expenditure the average figure of 24.8 calories per hour shows that washing table dishes is to be classed as moderate work, about midway between sewing and sweeping and washing. Somewhat more heat would no doubt be eliminated with heavier dishes (large platters, kettles, etc.) or ones which require harder rubbing than was given in these tests.

As regards the differences in the energy expended at tables of different heights,the figures show 21 calories per hour when the subject worked in a comfortable position, 25 calories when the pan was set so that her arms were raised during work, and 30 calories when she was obliged to bend over. It is hoped in the future to develop such experiments so that the oncome and effect of fatigue may be noted when similar tasks are performed under varying conditions.

SUMMARY

Fifty-three experiments on energy elimination during the performance of various household tasks were made, using a specially designed respiration calorimeter and a young woman subject. The results for such light tasks as sewing, crocheting, knitting, darning and embroidering, gave an average expenditure of 9 calories per hour more than that of the same subject sitting quietly in a chair; other tasks regarded as "harder work" than sewing, such as washing, sweeping and scrubbing

floors, caused an increased energy expenditure over the expenditure when at rest with the same subject, of about 50 calories per hour. Several other tasks studied gave results between these two figures: thus, ironing, dressing a child (life-size model) and dishwashing each requiring about 24 calories per hour.

During the experiment with dishwashing the height of the table used was varied, and a corresponding variation in energy expenditure was noted, a variation of 15 per cent in height of table causing an increase in energy expenditure of 20 per cent to 40 per cent. The observed increase of heat elimination well illustrates the importance of choosing equipment to "fit" the worker.

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